DETERMINATION OF THE REWARDING CAPACITY OF EDIBLE AND INJECTED Δ9-TETRAHYDROCANNABINOL IN ADOLESCENT AND ADULT MICE

by

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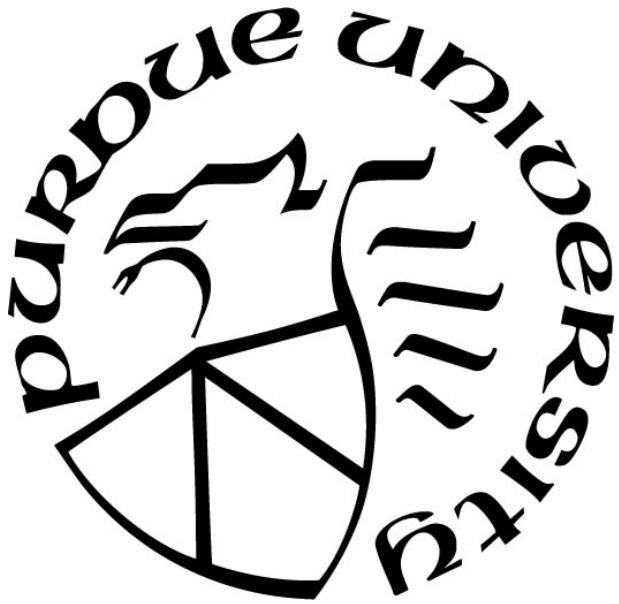

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ABSTRACT

Cannabis (and its main psychoactive component, THC) is one of the most widely-used drugs in the world, and recent expansion of its legal status has made it available in a variety of formulations and at a potency unrivaled in history. While its medicinal properties are gaining scientific support, so too is its potential to lead to abuse and dependence. Both initiation of cannabis use and frequent cannabis use are most prevalent in adolescence, and compared to adults, cannabis use by adolescents is associated with a greater likelihood of developing cannabis dependence and cannabis use disorder. Given the ethical limitations surrounding research that provides cannabis to non-users or non-adults, animal models of drug use can be valuable tools for the study of causes and consequences related to drug use, as well as allowing for investigating brain mechanisms underlying these factors. However, only recently have models in which animals reliably use cannabis (THC) at levels above its respective vehicle and at levels which produce consistent behavioral and physiological effects become available, and in no case has age-related differences in this use been examined. Thus, one goal of the current study was to directly compare the self-administration of edible THC (a route of administration used by humans and a formulation increasing in popularity) between adolescent and adult mice.

Adolescents also appear to be differentially sensitive to various effects of several classes of drugs, and they have been shown to be less sensitive to the aversive effects of cannabis, thereby putting them at greater risk for elevated and continued use. Evidence also suggests that, in addition to the risk associated with adolescent cannabis use, having initial positive subjective experiences resulting from its use is a strong predictor of subsequent cannabis dependence. Thus, the second goal of the current study was to use the place conditioning paradigm to examine the reward- (or aversion-) inducing properties of THC in adolescent and adult C57BL/6J mice, using both the traditional experimenter-administered THC (via injection) as well as edible THC self-administration.

Prior to initiating these THC studies, sensitivity of the place conditioning procedure to age-related differences in drug-induced reward was validated using cocaine, yielding locomotor stimulation in both ages and a decreased sensitivity to cocaine's rewarding properties in adolescent mice. When provided limited access to edible THC dough in doses ranging from 0.0

to 6.0 mg/kg, mice showed a dose-dependent reduction in consumption across access sessions, and this reduction was more rapid in adult mice at the highest doses, suggesting that adolescent mice might have been less sensitive to its aversive properties. These same mice, as well as a separate group of mice receiving injection (also 0.0 to 6.0 mg/kg THC), were given place conditioning sessions, alternating between THC dough and control dough or THC injection and vehicle injection, for 6 days per week and were tested once per week across a total of 3 weeks. Mice conditioned using edible THC showed a neutral response (neither reward nor aversion) at all doses. However, mice conditioned using injected THC showed a conditioned place aversion to the highest dose, which was more pronounced in adult mice. Interestingly, in mice self-administering edible THC, the dose of THC consumed was related to the outcome of place conditioning, such that a conditioned place preference was observed for adult mice which shifted their consumption of 3.0 mg/kg edible THC downward relative to those mice with full consumption of 3.0 mg/kg, and for adolescent mice which had the highest degree of consumption of 6.0 mg/kg edible THC relative to those mice with the lowest consumption of 6.0 mg/kg. Furthermore, initial place preference outcomes at the individual level at test 1 predicted subsequent doses of edible THC consumed, suggesting mice adjust their self-administration of edible THC based on the subjective experience it produces. Besides its impact in place conditioning, THC also had differential effects on body weight and locomotor activity based on age and route of administration. Collectively, this project demonstrates that adolescent mice are less sensitive to the hedonic properties of both cocaine and THC, and that differences in edible THC self-administration between ages, and between individuals within an age, are likely related the subjective experience of its rewarding and aversive properties.

INTRODUCTION

Cannabis Use

Humans have had a relationship with the cannabis plant for millennia, most notably for its use as medicine, food, fiber, and for intoxication [NASEM, 2017; Russo, 2007]. However, relatively recently its recreational use for intoxication has become much more prevalent [NASEM, 2017; Russo, 2007]. The growing popularity of the use of cannabis for recreational purposes is reflected in the increase in both its legal status and the variety of commercially available formulations. In the United States, nearly 50% of all students have tried cannabis by the end of high school, and 7.6% of young adults report daily use [Schulenberg, 2017]. Over half of the states in the United States have legalized or decriminalized recreational use of cannabis, and the majority of states allow for its medicinal use. Although it has multiple medicinal applications, including the domains of chronic pain, chemotherapy-induced nausea, and spasticity in multiple sclerosis, 90% of cannabis use is primarily for recreational purposes [NASEM, 2017].

Traditional use of cannabis involves smoking plant material (flower buds), and to a lesser extent, plant resin (hash). More recent formulations include oil-based extractions (concentrates) and food/drink products (edibles). While the cannabis plant contains over 100 phytocannabinoids, its psychoactive effects are due primarily to Δ^9-tetrahydrocannabinol (THC) [NASEM, 2017; Pertwee, 2008]. Not only do modern cannabis formulations (e.g. concentrates) typically contain substantially higher THC concentrations than does cannabis plant material, THC concentrations have increased significantly across formulations due to selective breeding of plants and refined extraction techniques [Chandra, 2019; NASEM, 2017]. Thus, with the capability for administration of THC, especially at extremely high levels, being greater than ever, research aimed at understanding the causes and consequences of this administration is as important as ever.

Animal Models of THC Use

Animal models have been valuable for elucidating mechanisms associated with various components of substance use and for predicting and evaluating potential treatments for substance use disorders. Substance use in animals typically comes in one of two flavors, experimenter-

administered or animal (self)-administered, but these are not mutually exclusive. While experimenter-administration of substances to animals provides the ultimate control over variables such as dose, timing, and conditions surrounding use, self-administration of substances by animals is better suited for investigating variables related to the use itself, such as motivation, reinforcement, and individual differences. Self-administration is usually operant or free-access in nature. Operant self-administration requires a response (i.e. lever press or nose poke) to obtain drug delivered intravenously, intracranially, in a fluid, or as vapor [Justinova, 2005; Koob, 1990; Vendruscolo, 2018; Zangen, 2006]. Free-access self-administration simply requires an animal to consume a drug in a fluid or food [Belknap, 1993; Green, 2008; Peris, 2006]. Given the right conditions, animals will readily self-administer the majority of drugs most frequently abused by humans, including alcohol, cocaine, opioids, and nicotine [Belknap, 1993; Bell, 2006; Ettenberg, 1982; Manzardo, 2002; Panlilio, 2007; Rose, 1997; Samson 1986]. Therefore, considering the prevalence of cannabis/THC use in humans, it is surprising that reliable THC self-administration in animals has proven difficult to demonstrate.

A number of early attempts failed to demonstrate reliable self-administration of THC in rodents or monkeys [Harris, 1974; Justinova, 2005; Lefever, 2014; Melis, 2017; van Ree, 1978], and those that were successful were complicated by other factors, like forced cannabinoid dependence, food deprivation, and previous drug self-administration experience [Tanda, 2000; Justinova, 2005; Melis, 2017]. More recently, the reinforcing capacity of THC via reliable self-administration at levels above its respective vehicle has been demonstrated intravenously in monkeys [Justinova, 2003; 2004], intracranially into discrete brain regions in rats [Zangen, 2006], intracerebroventricularly in rats [Braida, 2004], and intravenously in combination with cannabidiol (CBD) following forced exposure in rats [Melis, 2017; Spencer, 2018]. Notably, all of these studies required invasive surgery for implantation of catheters or cannulas. However, a surgery-free procedure demonstrating reliable, significant operant self-administration of THC-rich vapor by rats has very recently been described [Freels, 2020]. In addition to operant self-administration, models demonstrating reliable free-access oral self-administration of THC have been detailed in both mice [Leung, 2019; Smoker, 2019a; Smoker, 2019b] and rats [Barrus, 2018; Kruse, 2019; Nelson, 2018]. Studies employing free-access oral, or operant vapor, self-administration of THC have the advantages of using routes of administration (ROAs) analogous to those used by humans and of not requiring surgery, restraint, or food deprivation. However, in

all cases of oral self-administration, THC was provided in a palatable substance (i.e. cookie, dough, gelatin, or sweetened fluid) and reduced the consumption of that substance at higher doses. Thus, the reinforcing capacity of orally self-administered THC in the absence of a palatable vehicle isn't fully known. Taken together, THC has proven to have a relatively low capacity for reinforcement in comparison other popular recreationally-used drugs, or at least is only reinforcing under a more stringent set of parameters. This might suggest that animals do not find THC to be rewarding to the same degree, or in the same manner, that humans do.

Place Conditioning Paradigm

In addition to being self-administered, substances which are used recreationally by humans have been shown to be rewarding in animals using the place conditioning paradigm [Bardo, 2000; Tzschentke, 1998; Tzschentke, 2007]. The basic procedure involved in place conditioning pairs the experience of the effects of a drug (unconditioned stimulus, US) with a particular context (conditioned stimulus, CS+) and an analogous drug-free experience (non-US) with a different context (CS-). Following alternating pairings of US/CS+ and non-US/CS-, the relative time spent in the drug-paired context (CS+) can provide an indication of the rewarding (increased time) or aversive (decreased time) properties of a drug, known as conditioned place preference (CPP) or conditioned place aversion (CPA), respectively. This procedure has two particular advantages, the ability to assess a drug's motivational properties along a spectrum from aversion to reward and to assess a drug's motivational or approach-eliciting capacity in the absence of its acute pharmacological effects.

Place Conditioning with THC

Demonstrating the rewarding capacity of THC in animals via conditioned place preference has been less straightforward than for other drugs frequently abused by humans. THC place conditioning studies have been conducted in multiple strains of both mice [Burgdorf, 2020; Cheng, 2004; Ghozland, 2002; Han, 2017; Hutcheson, 1998; Kardash, 2020; Ponzoni, 2019; Soria, 2004; Valjent, 2000; Vann, 2008] and rats [DeVuono, 2017; Braida, 2004; Cheer, 2000; Hempel, 2016; Hempel, 2017; Le Foll, 2006; Lepore, 1995; Mallet, 1998; Parker, 1995; Quinn, 2008; Sanudo-Pena, 1997; Schramm-Sapyta, 2007; Wakeford, 2016; Young, 2017; Zangen, 2006] and generally support and inverted U-shaped THC dose response curve.

Specifically, low THC doses tend to produce reward (CPP), while high doses tend to produce aversion (CPA), and intermediate (or extremely low) doses tend to be neutral. However, some studies fail to detect any effect. Furthermore, the dose response curve has been shown to be affected by parameters such as dosing interval [Lepore, 1995], THC pre-exposure [Ghozland, 2002; Valjent, 2000], nicotine pre-exposure [Ponzoni, 2019], presence of other cannabinoids [Vann, 2008], and age [Quinn, 2008; Schramm-Sapyta, 2007]. Taken together, place conditioning studies using THC indicate that its rewarding capacity is low relative to other recreationally-used drugs, in line with its relatively low reinforcing capacity as determined by self-administration studies.

The Endocannabinoid System

The brain's endogenous cannabinoid (endocannabinoid, eCB) system is composed of a unique set of receptors as well as ligands and their synthesis and degradation machinery. eCB receptors include both cannabinoid-type-1 (CB1R) and cannabinoid-type-2 (CB2R), with CB1R widely expressed in the central nervous system and CB2R mainly expressed in the immune system [Castillo, 2012; Kano, 2009]. THC is a partial agonist at both CB1Rs and CB2Rs and also possesses actions at other receptors, including the G-protein-coupled receptor GPR55 and transient receptor potential vanilloid receptor 1 (TRPV1) [Pertwee, 2008]. However, its psychoactive properties are due to its interaction with the eCB system, mediated primarily through CB1Rs [NASEM, 2017; Pertwee, 2008]. The eCB ligands 2-arachidonylglycerol (2-AG) and anandamide act as a CB1R agonist and partial agonist, respectively. These eCBs are released from postsynaptic cells to bind to CB1Rs, activation of which attenuates presynaptic neurotransmitter release [Kano, 2009; Katona, 2008]. eCB production is typically on-demand (activity dependent), but tonic eCB signaling can also occur [Castillo, 2012]. Following transport into the presynaptic cell, eCB signaling is terminated via enzymatic degradation of 2-AG predominantly by monoacylglycerol lipase (MAGL) and of anandamide by fatty acid amide hydrolase (FAAH) [Freund, 2003; Kano, 2009].

Neurobiology of Reward

The most highly-implicated substrate underlying the rewarding properties of drugs of abuse is the activation of the mesolimbic dopamine (DA) pathway, specifically DA

neurotransmission in the ventral striatum (nucleus accumbens, NAc) from projections originating in the ventral tegmental area (VTA) [Bardo, 1996; Cheer, 2007; Doremus-Fitzwater, 2016; Maldonado, 2006; Murray, 2010; Ranaldi, 2014, Tanda, 2003; Tanda, 2016; Tirelli, 2003]. VTA DA cells respond to the experience of natural rewards (e.g. food, sex), and increased levels of extracellular DA in the NAc are associated with reward-seeking behavior [Cheer, 2007; Mohebi, 2018; Ranaldi, 2014; Rodriguez-Manzo, 2020]. In addition, multiple classes of abused drugs produce increases in NAc DA levels by various mechanisms, for example by preventing the removal of NAc DA from the extracellular space via blockade of its reuptake (psychostimulants) or by increasing the activity of VTA DA cells via suppression of their tonic inhibition (opioids) [Cheer, 2007; Tanda, 2003]. VTA DA cells respond to both rewards and novel stimuli, producing increased NAc DA release; however, the response to stimuli habituates if not reward-paired [Ranaldi, 2014]. Following conditioning, a stimulus paired with reward can elicit the same neural response as the primary reward (VTA DA cell activation and NAc DA release) as well as a similar motivational state [Ranaldi, 2014]. Such would be expected to be the case with rewarding, drug-paired contexts in the place conditioning paradigm. That is, following conditioning, an increase in both VTA DA cell activity and NAc DA release in response to the CS+ should underlie the motivational state associated with approach behavior, resulting in CPP. Accordingly, calcium signals (indicative of elevated neural activity) increase in both the VTA and its terminals in the NAc on cocaine place conditioning test sessions prior to entry into the cocaine-paired, but not saline-paired, context, and the magnitude of calcium increase is associated with the magnitude of cocaine-induced CPP [Calipari, 2017]. Furthermore, amphetamine- and heroin-induced conditioned place preferences can be disrupted via intra-NAc DA receptor antagonism and/or 6-hydroxydopamine lesions of the NAc [Bardo, 1996; Tirelli, 2003].

NAc DA release can serve as an activational signal for decisions to perform motivated action [Berke, 2018; Mohebi, 2018]. However, while phasic NAc DA release from VTA DA cells modulates this signal, it can also be influenced by the basolateral nucleus of the amygdala (BLA) independently of the VTA, as well as influenced locally by NAc cholinergic interneurons [Berke, 2018]. Therefore, there is not perfect correspondence between VTA DA cell activity and NAc DA release. For example, while NAc (and PFC) DA release has been shown to be associated with reward rate in a probability-based task, VTA DA cell activity remained unrelated

[Mohebi, 2018]. While tonic VTA DA cell activity has very little impact on NAc DA release, it might provide the basal tone for fluctuations related to stimulus-outcome associations [Berke, 2018]. The activity of VTA DA cells increases in response to unexpected stimuli and unexpected outcomes (rewards or punishments) [Berke, 2018; Cohen, 2012; Matsumoto, 2016; Mohebi, 2018]. In addition, when stimuli come to reliably predict outcomes (become cues), VTA DA cell activity shifts from responding to the outcome to responding to these cues, resulting in a relative increase or decrease in activity for cues predicting rewards or punishments, respectively [Cohen, 2012; Matsumoto, 2016; Mohebi, 2018]. However, when outcomes predicted by cues are omitted, VTA DA cells respond in the opposite direction, a relative decrease or increase in activity for expected rewards or punishments, respectively [Matsumoto, 2016; Cohen, 2012]. Thus, an important role for VTA DA signaling is in value prediction error, the discrepancy between the predicted and experienced value of outcomes. This function provides necessary information to the NAc in service of valuation of resource allocation [Berke, 2018; Mohebi, 2018], as would be expected for motivated action related to reward-paired contexts in the place conditioning paradigm.

Role of CB1R in Reward

Although the results of both self-administration and place conditioning studies using THC have been inconsistent, there is much evidence for a role of the eCB system, specifically CB1R, in reward. Synthetic CB1R/CB2R agonists, CP55940, WIN552122, and JWH018, all of which display greater CB1R affinity and efficacy than THC, are self-administered intravenously or intracerebroventricularly by rats and/or mice [Tanda, 2016], and this self-administration can be blocked by administration of the CB1R inverse agonist SR141716A [Braida, 2001b; De Luca, 2015; Lefever, 2014; Martellotta, 1998; Spano, 2004]. In addition, although synthetic CB1R agonists typically produce CPA in rodents [Tanda, 2016], CPP produced by CP55940 in rats can be blocked by SR141716A administration [Braida, 2001a]. Evidence for the localization of the reinforcing and rewarding effects of CB1R agonism was demonstrated in a set of studies using intracerebral administration of THC into discrete brain regions, either as operant self-administration or as administered by experimenters in the context of place conditioning [Zangen, 2006]. Rats showed reliable THC self-administration in both the VTA and NAc shell (NAcSh), but not in the NAc core, substantia nigra, or region dorsal to the VTA, and THC also produced

CPP when administered in either the posterior VTA or NAcSh (not anterior NAcSh). Thus, THC was found to be reinforcing/rewarding only when administered in discrete regions within the mesolimbic DA pathway, and in the case of self-administration, required CB1R signaling.

That THC can exert rewarding effects when localized within the VTA and NAc suggests an important reward-related role for CB1R activity in these regions. CB1Rs are located in both the VTA and NAc at relatively low density [Herkenham, 1990; Herkenham, 1991], and they are not located on principal projection cells (i.e. VTA DAergic cells, NAc GABAergic medium spiny neurons (MSNs)) in either region [Zlebnik, 2016]. Instead, CB1Rs are located presynaptically, primarily on GABAergic, and also on glutamatergic synapses, on VTA DA cells [Han, 2017; Kano, 2009; Maldonado, 2006]. In the NAc, CB1Rs are located presynaptically on GABAergic and glutamatergic inputs as well as GABAergic interneurons, all of which synapse with NAc GABAergic MSNs, which themselves project to VTA DA cells [Maldonado, 2006; Wright, 2017; Zlebnik, 2016]. In addition, CB1Rs are located presynaptically on GABAergic cells in other reward-related brain regions, including the hippocampus and BLA, which inhibit glutamatergic projections to VTA DA cells [Maldonado, 2006].

Collectively, CB1Rs are positioned to modulate the activity of VTA DA cells directly, within the VTA, or indirectly, by modulating the activity of projections to VTA DA cells from the NAc and other regions, and the effect of systemic CB1R activation is typically a shift towards disinhibition of VTA DA cells. Thus, administration of THC, or the synthetic CB1R agonists CP55940 or WIN552122, results in increased firing of VTA DA cells and increased NAc DA release [Murray, 2010; Scherma, 2016; Spano, 2010; Tanda, 1997; Tanda, 2003; Zlebnik, 2016], an effect which is blocked by CB1R antagonism [Tanda, 1997; Cheer, 2007]. This enhanced phasic activity of VTA DA cells should have the capacity to impact value prediction error signals when occurring in reward- (or aversion-) related contexts. CB1R-mediated disinhibition of VTA DA cells is due primarily to a decrease in presynaptic GABA release, resulting in a decrease in inhibitory postsynaptic currents [Cheer, 2007; Murray, 2010]. In addition to VTA DA cells, VTA GABA cells also play a role in value prediction error, in this case by increasing activity to aversive outcomes and during the delay between cue and outcome in a reward magnitude-related manner [Cohen, 2012]. Taken together, exogenous activation of CB1Rs has the capacity to exert a pronounced modulatory effect on mesolimbic DA pathway

signaling, potentially interfering with value prediction error and biasing it towards a pattern of activity underlying reward-related behavior.

The consistency with which exogenous cannabinoid challenges impact mesolimbic DA transmission suggest a role for the eCB system in reward. Indeed, activation of VTA DA cells results in eCB release and retrograde signaling to presynaptic GABAergic and glutamatergic inputs to modulate inhibitory/excitatory balance [Maldonado, 2006; Zlebnik, 2016], and this is seen following both natural (e.g. copulation) and drug (e.g. cocaine) rewards [Rodriguez-Manzo, 2020; Zlebnik, 2016]. Much evidence suggests that CB1R functioning is implicated in, and previous cannabinoid exposure tends to enhance, the rewarding/reinforcing properties of many classes of drugs of abuse, including ethanol, nicotine, and opioids [Maldonado, 2006; Panlilio, 2018; Tanda, 2003]. However, the evidence for the role of CB1Rs in cocaine reward/reinforcement has been mixed. For example, congenital deletion of CB1Rs in rodents has been shown to both impact and have no effect on cocaine self-administration, and similar mixed results have been obtained for the effect of SR141716A on cocaine-induced increases in NAc DA release [Cheer, 2007; Maldonado, 2006; Panlilio, 2018; Soria, 2005; Tanda, 2003]. Much of the discrepancy can likely be attributed to the direct impact of psychostimulants on NAc DA levels by preventing reuptake, unlike other classes of drugs. Methodological differences contribute as well, as SR141716A has been shown to prevent the increase in transient NAc DA release in response to cocaine when measured with greater temporal precision (voltammetry vs microdialysis), in line with the effect of SR141716A on the NAc DA response to other drugs and natural rewards [Cheer, 2007; Melis, 2007]. Mounting evidence suggests that the eCB system, specifically through modulation of VTA DA cell activity, plays an integral role in the rewarding properties of drugs of abuse, including THC, as well as natural rewards.

THC Aversion

When considering just the role of the eCB system, and specifically CB1Rs, in mechanisms underlying reward, the limited evidence for rewarding effects of THC in non-human animals is puzzling. However, there is substantial evidence that THC is capable of eliciting aversive states in both humans and non-human animals. Apart from medicinal benefits, humans report positive effects of cannabis use including euphoria, relaxation, and a pleasant subjective state [Lile, 2013; Mokrysz, 2016; NASEM, 2017; Patel, 2009]. Yet acute use is also associated

with a number of negative subjective effects, often co-occurring in individuals reporting positive effects, including increased anxiety, paranoia, and psychotic symptoms as well as impairment in cognitive and attentional capabilities [Ballard, 2011; Battacharyya, 2010; Battacharyya, 2017; D'Souza, 2004; Hanson, 2010; Lile, 2013; Mokrysz, 2016; NASEM, 2017; Patel, 2009]. These negative effects aren't simply a product of laboratory settings, as increases in anxiety and psychotic symptoms also occur when cannabis users smoke their own cannabis in their own homes [Morgan, 2010]. Despite the occurrence of these negative effects, cannabis users continue to use the drug.

In line with human reports, studies using rodents also demonstrate THC's capacity to induce aversive states. When paired with palatable foods/liquids or with distinct contexts, THC administration is able to support both conditioned taste aversion [Barrus, 2018; Parker, 1995; Schramm-Sapyta, 2007; Wakeford, 2016] and conditioned place aversion [Cheer, 2000; DeVuono, 2017; Ghozland, 2002; Han, 2017; Hutcheson, 1998; Lepore, 1995; Mallet, 1998; Parker, 1995; Quinn, 2008; Sanudo-Pena, 1997; Schramm-Sapyta, 2007; Soria, 2004; Vann, 2008]. In addition, orally self-administered THC appears to be capable of producing conditioned taste aversion in rodents, indicating that THC has aversive properties even in the absence of stress associated with forced exposure [Barrus, 2018; Kruse, 2019; Nelson, 2018; Smoker, 2019b]. One consistent component of this aversive state in rodents appears to be anxiety, as it is fairly reliably induced by THC in rodent models of anxiety-like behavior, such as the elevated plus maze or light/dark box, in both mice [Kasten, 2017; Kasten, 2019; Onaivi, 1990; Patel, 2006] and rats [Onaivi, 1990; Schramm-Sapyta, 2007]. However, an anxiolytic effect of THC has been demonstrated following administration of relatively low doses [Berrendero, 2002; Rubino, 2007], comparable to those producing a lack of effect or CPP in the place conditioning paradigm.

As with THC-induced reward, CB1Rs also play an integral role in both THC's aversive properties and its impact on anxiety. In humans, therapeutic use of the CB1R inverse agonist SR141716A (Rimonabant) is associated adverse side effects, including increased anxiety [Patel, 2009]. In rodents, evidence is mixed with regard to the effect of CB1R signaling on anxiety. Synthetic CB1R agonists as well as CB1R antagonists/inverse agonists have been shown to possess both anxiogenic and anxiolytic properties [Kano, 2009; Patel, 2006; Patel, 2009], and place conditioning with synthetic CB1R agonists typically produces CPA [Carvalho, 2016;

Tanda, 2003]. Furthermore, the anxiolytic effects of THC can be blocked by SR141716A or the CB1R antagonist AM251 [Berrendero, 2002; Rubino 2007]. Enhancing eCB signaling through FAAH inhibition (URB597) or combination FAAH/eCB uptake inhibition (AM404) in rodents is also anxiolytic [Kathuria, 2003; Patel, 2006; Patel, 2009], an effect which can be blocked by SR141716A [Kathuria, 2003]. Therefore, eCB signaling through CB1Rs is likely involved in modulation of unconditioned anxiety. As a partial CB1R agonist, THC should have the capacity to facilitate or impede eCB signaling as a complement to, or in competition with, endogenous CB1R ligands, respectively, providing some explanation for its bi-directional effects on anxiety-like behavior.

The substrates underlying THC's aversive properties appear to overlap only partially with the mesolimbic DA pathway, which plays an important role in its rewarding properties. THC administration in humans alters activity in a number of brain regions including midbrain and striatum as well as the prefrontal cortex (PFC), amygdala, and hippocampus [Bhattacharyya, 2017; Bossong, 2012], all regions containing CB1Rs [Herkenham, 1990; Herkenham, 1991]. Within mesolimbic circuitry, VTA CB1R antagonism via AM251 produces CPA, an effect which is blocked by NAcSh DA receptor antagonism [Ahmad, 2017]. This is consistent with reductions in NAc DA being associated with aversive states [Tanda, 2003], as a relative decrease in VTA DA signaling via AM251 is aversive, but complete lack of DA signaling in the NAcSh prevents this aversion [Ahmad, 2017]. On the contrary, VTA CB1R agonism via CP55940 produces CPP [Ahmad, 2017], consistent with role of CB1R activation on VTA DA cell firing. However, this effect is blocked by BLA DA receptor antagonism, implicating a role for the BLA in hedonic processing. Finally, THC-induced CPA in mice can be attenuated by knock-out of CB1Rs on subcortical glutamatergic cells (including VTA) [Han, 2017]. This suggests opposing roles for VTA DA cell afferent signals, with CB1R-mediated inhibition of GABAergic signaling contributing to reward and CB1R-mediated inhibition of glutamatergic signaling contributing to aversion.

In humans, THC increases subjective anxiety as well as both the physiological response and amygdala activation to fearful stimuli [Bhattacharyya, 2017]. Furthermore amygdala CB1R availability is associated with subjective reports of anxiety [Bhattacharyya, 2017], suggesting a role for amygdala CB1R signaling in mediating aversive states. This is supported by rodent literature indicating that THC has anxiogenic effects when administered in the BLA but has

anxiolytic effects when administered in the PFC or ventral hippocampus, all of which can be blocked by AM251 [Rubino, 2008]. Additionally, the rewarding effects of systemically administered morphine can be enhanced by CB1R antagonism in the BLA or PFC or can be shifted to aversion by CB1R agonism in the BLA or PFC [Ahmad, 2013; Ahmad, 2016]. Thus, CB1R signaling in regions associated with emotional processing outside of the mesolimbic DA pathway, such as the amygdala, PFC, and hippocampus, has the ability to modulate reward and aversion and likely underlies some of THC's aversive properties.

Role of the Endogenous Opioid System

The eCB system interacts with a number of brain neurotransmitter systems, but with respect to cannabinoid drug self-administration, reward, and aversion, its interaction with the endogenous opioid system appears to be particularly important [Maldonado, 2006; Spano, 2010; Tanda, 2003]. For example, opioid receptor antagonism via naloxone or naltrexone reduces cannabis/THC self-administration by rodents, monkeys, and humans and also prevents THC- or WIN552122-induced increases in NAc DA release and THC-induced CPP [Braida, 2004; Haney, 2015; Justinova, 2004; Tanda, 1997]. Furthermore, heroin self-administration by rats alters CB1R and mu-opioid receptor density and functioning in a number of brain regions involved in reward- and emotion-related processing [Fattore, 2007]. Many of the effects are bidirectional, such that pharmacological or genetic manipulations targeting CB1Rs can impact opioid self-administration and reward-related behaviors as well as mu-opioid receptor density and functioning [Fattore, 2007; Maldonado, 2006; Spano, 2010]

Within the endogenous opioid system, there appears to be a distinction between receptor subtypes in terms of their impact on the rewarding or aversive properties of THC. Specifically, evidence suggests that THC's rewarding and aversive properties are mediated by mu-opioid and kappa-opioid receptors, respectively. In human cannabis users, the positive subjective effects of smoked cannabis are reduced by naltrexone, an antagonist with the greatest affinity for mu-opioid receptors [Haney, 2015]. In rodents, CPP induced by a low dose of THC is absent in mu-opioid receptor knock-out mice, while CPA induced by a higher dose of THC is absent in kappa-opioid receptor mice [Ghozland, 2002]. Likewise, in rats, CPP induced by infusion of CPP55940 into the VTA is prevented by mu-opioid receptor antagonism, while CPA induced by infusion of AM251 into the VTA is prevented by kappa-opioid receptor antagonism [Ahmad,

2017]. As THC's impact on anxiety likely contributes to its rewarding and aversive capacity, it is not surprising that the mu/kappa distinction in anxiety parallels that in reward/aversion. For example, the anxiolytic effects of THC can be blocked by mu- or delta- (but not kappa-) opioid receptor antagonism [Berrendero, 2002; Kano, 2009], while the anxiogenic effects of THC or CP55940 can be blocked by kappa- (but not mu-) opioid receptor antagonism [Kano, 2009; Onaivi, 1990]. Interestingly, evidence suggests that THC's rewarding and aversive properties operate concurrently and are in competition for behavioral control, as either antagonism or congenital deletion of kappa-opioid receptors is capable of unmasking CPP to low doses of THC which were otherwise shown to be neutral or to induce CPA [Cheng, 2004; Ghozland, 2002].

Combination of Reward and Aversion

It is clear that THC has both rewarding and aversive properties. These properties appear to be related to CB1R activity in multiple, but only partially overlapping, brain regions. Among others, these include the VTA, which plays a critical role in THC-induced reward, as well as the amygdala and PFC, which modulate reward and play an integral role in anxiety as a component of THC-induced aversion. Interestingly, individual VTA DA cells can respond to both reward- and aversion-related cues and to omitted outcomes (prediction error), and these cue and error responses are related within a valence (reward or aversion) but not between valences. In addition, the increased response to reward cues occurs earlier than the decreased response to aversion cues, and the response to mixed cues (predicting both outcomes) is an increase followed by a decrease [Matsumoto, 2016]. Taken together, this suggests that reward- and aversion-related signals might be distinct inputs to VTA DA cells [Matsumoto, 2016], providing a substrate for CB1R-mediated modulation of each. In addition, THC's rewarding and aversive properties receive a differential contribution from opioid receptors based on subtype. This suggests that THC's hedonic capacity isn't simply a shifting subjective state on a single spectrum with extremes at reward and aversion, but rather that the subjective state induced by THC is composed of competing yet partially overlapping systems. Indeed, THC is capable of eliciting both positive and negative subjective effects in the same individuals within the same session [Haney, 2007; Lile, 2013; Mokrysz, 2016]. While humans can report multiple valences in experimental settings, a major disadvantage of non-human research is that measurement of an animal's behavior can really only provide the net effect of a drug, positive, negative, or neutral.

However, that pharmacological or genetic manipulations in mice can reveal the rewarding effects of THC under conditions and at doses which otherwise produce aversion or neutrality [Cheng, 2004; Ghozland, 2002] suggests that rodents likely experience both valences concurrently. An intriguing possibility then is that individuals might not simply find THC rewarding or aversive, but might have varied subjective experiences containing elements of each valence. Thus, individual sensitivity to THC-induced reward, aversion, or the balance of the two, could contribute to individual differences in susceptibility to the development of problematic cannabis use.

Adolescent Cannabis Use

The period of adolescence does not have rigidly defined borders in terms of age. In humans, adolescence is considered to be approximately the range of 10-19 years of age [Thorpe, 2020]. In rodents, a conservative age range for adolescence is PND 28-42, and at the broadest it can be considered to cover the entire period of development from PND 21-60 [Laviola, 2003; Spear, 2000; Thorpe, 2020]. Human adolescence is a developmental period marked by numerous behavioral changes, including increases in risk-taking and social interaction as well as cognitive development, and this is true for other species such as rodents and non-human primates [Spear, 2000; Thorpe, 2020]. In addition, the recreational use of drugs increases during this period [Laviola, 2003; Spear, 2000; Thorpe, 2020], with half of adolescents having used illicit drugs by the 12^{th} grade [Schulenberg, 2017]. Approximately half of individuals have tried cannabis prior to the age of 20, a proportion which increases relatively little in subsequent years, and the proportion of individuals reporting daily cannabis use peaks before the age of 25 [Schulenberg, 2017]. Thus, both the initiation of cannabis use and heavy cannabis use are most common in adolescents or very young adults.

Compared to adults, cannabis use by adolescents is associated with a number of cannabis-related negative outcomes, including higher rates of cannabis use disorder and faster development of cannabis dependence [Clark, 1998; Forman-Hoffman, 2017], with the latter marked by the development of tolerance to cannabis and withdrawal effects (e.g. changes in mood, appetite, or physiological symptoms) following cessation of its use [American Psychiatric Association, 2013]. One factor that might contribute to this age-related discrepancy in cannabis use outcomes is the subjective experience of cannabis intoxication. Following acute cannabis

vapor exposure, adolescents have been shown to be less sensitive to many negative subjective effects (e.g. dry mouth and anxiousness), to be less cognitively impaired, and to display an increased desire for additional cannabis when compared to adults [Mokrysz, 2016]. This differential subjective sensitivity could contribute to vulnerability to problematic cannabis use, as both endorsing positive effects of and having initial positive reactions to cannabis use in adolescence are associated with cannabis dependence [Fergusson, 2003; Le Strat, 2009; Zeiger, 2010]. Although degree of cannabis use and experiencing positive effects following use are biased towards adolescence in humans, the overwhelming majority of self-administration and place conditioning studies have been conducted in adult animals. Of the few studies examining THC self-administration in adolescent animals, none have compared levels of use to that of adult animals [Kruse, 2019; Nelson, 2018; Smoker, 2019a]. However, a limited number of THC place conditioning studies have made direct age comparisons and tend to corroborate the age-dependent differential sensitivity to THC found in humans, as doses of THC which are aversive for adult rats are not so [Quinn, 2008], or less so [Schramm-Sapyta, 2007], for adolescent rats. Thus, one factor contributing to THC's relatively low reinforcing and rewarding capacity in animal research might be the assessment of these effects in adulthood, a later developmental stage than initial THC exposure typically occurs in humans, and one that appears to confer greater sensitivity to THC's aversive properties.

Adolescent Neurobiology

In conjunction with the prominent behavioral changes characteristic of adolescence, there are a number of neurobiological features of this developmental period which are relevant to the experience and consequences of drug use. Adolescence is a period of marked limbic and cortical development, including synaptic pruning and reorganization, and development of the amygdala and PFC continues into young adulthood [Lee, 2012; Thorpe, 2020]. Compared to adults, adolescents differ in elements of mesolimbic DA signaling, including being the time of peak striatal DA receptor density, having increased DA storage pool but decreased release in the NAc, and having enhanced tonic and burst firing of VTA DA cells [Doremus-Fitzwater, 2016; Laviola, 2003; Thorpe, 2020; Tirelli, 2003]. Thus, adolescents differ from adults in the composition and function of some brain regions and systems responsible for processing reward and emotion. In addition, a number of developments in the eCB system take place during

adolescence. While the eCB system is present as early as gestational day 11 [Lee, 2012], CB1R density and binding are low early in development but rise to a peak in adolescence, which plateaus or decreases into adulthood in limbic, striatal, and midbrain regions [Doremus-Fitzwater, 2016; Lee, 2012; Thorpe, 2020]. This developmental trajectory is similar for eCB ligands and some forms of eCB function (e.g. retrograde inhibition of pre-synaptic neurotransmitter release) [Doremus-Fitzwater, 2016; Lee, 2012; Thorpe, 2020]. The changes taking place in reward- and aversion-relevant brain systems during adolescence, including the eCB system, might alter the response to, or consequences of, challenges to CB1R signaling via exogenous compounds. Neurobiologically, adolescent exposure to THC or CP55940 leads to alterations in both CB1R and mu-opioid receptor density and function in the VTA, NAc, and/or PFC [Biscaia, 2008; Ellgren, 2007; Kruse, 2019; Thorpe, 2020]. Furthermore, some consequences of THC exposure, like VTA DA cell hyperactivity, are specific to adolescence (vs adulthood) [Renard, 2017]. Behaviorally, compared to adults, adolescents show a shift in the balance of reward and aversion induced by THC [Mokrysz, 2016; Quinn, 2008; Schramm-Sapyta, 2007] or WIN552122 [Carvalho, 2016]. Taken together, adolescence represents a period of unique neurobiological development, differential sensitivity to THC, and protracted neurobiological and behavioral consequences of THC exposure, providing a strong incentive for preclinical investigations of THC targeting this stage of development.

Rationale

Although cannabis is one of the most popular recreationally-used drugs by humans, animal models of THC self-administration fail to adequately model that use. Furthermore, place conditioning in animals has been fairly inconsistent with respect to the rewarding and aversive capacity of THC. Finally, initiation of cannabis use, daily cannabis use, and negative outcomes associated with cannabis use are most pronounced in adolescence in humans. However, very few preclinical studies employ adolescent animals for THC self-administration or place conditioning procedures, and no study has combined these two procedures within the same animals. Therefore, I sought to combine a mouse model of edible THC self-administration with a version of THC place conditioning within the same animals to examine the impact of age (adolescent vs adult) on THC self-administration, THC reward/aversion, and the relationship between the two. Given the novelty of this project, a complementary assessment of THC place

conditioning was conducted in adolescent and adult mice using the more traditional route of administration, i.p. injection. THC doses and experimental timing for these procedures were chosen based on THC place conditioning literature and previous data collected using the mouse edible THC model. Additional measures, including locomotor activity, body weight, and fecal boli, which are known to be affected by THC administration, were included to examine the impact of age and ROA and to verify the efficacy of administered THC.

In contrast to THC, cocaine is very reliable in its capacity to support self-administration and CPP in rodents, and it is often used as a positive control for other manipulations in these assays. Given that THC place conditioning had not been conducted in our lab and that I had personally not conducted place conditioning of any kind, an initial place conditioning study using cocaine was conducted as a positive control. Adolescent rodents are generally less sensitive than adult rodents to cocaine's acute effects on locomotor activity and its rewarding properties. Therefore cocaine doses and experimental timing were chosen to maximize sensitivity between age groups in preparation for subsequent assessments using THC.

Hypotheses

Regarding cocaine place conditioning, it was hypothesized that C1) cocaine would produce CPP and C2) acute locomotor stimulation in adult mice, and that C3) these effects would be attenuated or absent in adolescent mice. Regarding edible THC self-administration, it was hypothesized that T1) mice would consume edible THC across multiple sessions, but that T2) THC would cause a dose-dependent reduction in consumption, due to its post-ingestive aversive properties. Furthermore, it was hypothesized that T3) adolescent mice would achieve higher THC doses than adult mice. Regarding THC place conditioning, it was hypothesized that T4) edible THC would produce CPP which would be T5) exclusive to adolescent mice or observable at higher THC doses in adolescent vs adult mice. In addition, it was hypothesized that T6) injected THC would produce CPA at the highest dose, and that T7) the gradient of place conditioning outcomes (preference → neutral → aversion) would be shifted rightward across THC doses for adolescent vs adult mice.

MATERIALS AND METHODS

Drugs

Cocaine was purchased from Sigma Aldrich (St. Louis, MO) and dissolved in 0.9% physiological saline. Cocaine solutions were prepared daily, and the lower dose was always diluted from the higher dose. Cocaine/saline injections were given at 10 ml/kg using a 27 gauge needle. THC was provided by the National Institute on Drug Abuse (Bethesda, MD) dissolved in 95% ethanol at a concentration of 100 mg/ml. Edible dough was created by combining ingredients to produce a single batch of dough, from which individual servings were portioned per mouse based on weight. The THC-ethanol solution, or 95% ethanol alone, was dissolved in glycerol and combined with flour, sugar, and salt (ratio = 20ml:30g:4g:1g) to produce THC dough or control dough, respectively. For injection, the THC-ethanol solution, or 95% ethanol alone, was dissolved in a vehicle of Tween-20, 100% ethanol, and 0.9% physiological saline (ratio = 1:1:18) to produce injectable THC and vehicle solutions, respectively. For both edible and injected THC, ethanol concentration varied by dose but was equal for control/vehicle and THC preparations within a given dose and ROA. Mice receiving 0.0 g/kg THC received preparations containing an ethanol concentration equal to mice receiving 6.0 g/kg THC. All edible dough and injected solutions were prepared daily. Edible dough was provided at 5 g/kg in a clean, empty mouse cage with water, but not food, available. An illustration of dough preparation and serving is provided in (Figure 1). Mice were given access to edible dough for 30 minutes each day, except for the initial exposure (2 hours). THC/vehicle injections were given at 10 ml/kg using a 25 gauge needle.

Apparatus

All conditioning and testing took place in light and sound attenuating chambers measuring 53 x 58 x 43 cm (l x w x h). Within the chambers, mice were constrained within a plexiglass box measuring 25 x 14 x 15 cm, which rested atop metal grid and/or hole floor textures, and lights were turned off. An illustration of floor textures and arrangements is provided in (Figure 2). Great care was taken to ensure that adjoining floor textures were aligned at the center (lengthwise) of the plexiglass enclosure, creating 2 sides equal in area. Mouse activity and position was monitored by AccuScan VersaMax activity monitors (Omnitech

Electronics Inc., Columbus, OH) using infrared photocell beams spaced 2.5 cm apart. All measured parameters, including position (side) and distance travelled (cm), were collected after the completion of a given session and binned into 1-minute intervals regardless of session duration. Between sessions, floor textures were wiped with a damp sponge, and chamber floors were cleaned with Clidox-S (Pharmcal Research Laboratories, Inc., Naugatuck, CT). Plexiglass enclosures were cleaned prior to all baseline and testing sessions.

Cocaine Place Conditioning

A timeline of cocaine place conditioning is provided in (Figure 3). 56 mice were purchased from the Jackson Laboratory and arrived on postnatal day (PND) 21 or 56 for adolescents or adults, respectively. Upon arrival, all mice received an ear punch for designation and were pair-housed with an age-matched cage mate under a 12-hour, reverse light-dark cycle in a temperature- and humidity-controlled vivarium. Mice had ad libitum access to food and water, except when in conditioning chambers. Mice had an additional 4 days of habituation, including a single 2-hour habituation session in the testing room on PND 25 or 60. Beginning on PND 26 or 61 (experimental day 0), mice were weighed daily within 1 hour of the dark cycle onset and habituated to the testing room for 30 minutes prior to placement in conditioning chambers.

On day 0, mice received a saline injection immediately before a baseline floor preference assessment, which was 15 minutes in duration and used both floor textures in a sided-by-side arrangement. Floor texture side was counterbalanced within each age group. Prior to day 1, mice were rank-ordered by time spent on their non-preferred floor texture within each age group. A total of 8 mice were excluded from the remainder of the study, 1 adult mouse and 2 adolescent mice with the lowest times at baseline (and their cage mates), as well as 1 adult mouse which died prior to day 0 (and his cage mate). Cocaine conditioning doses (0, 1, or 5 mg/kg, n's = 8 per dose per age) were assigned to match groups based on time spent on the non-preferred floor during baseline.

A single cocaine place conditioning trial occurred across days 1-2, with one session per day. Mice received an injection and were placed in the conditioning chamber for 15 minutes on a single floor texture. Cocaine was paired with the non-preferred floor texture, and conditioning order (cocaine vs saline) was counterbalanced within age and dose. Cage mates received the

same cocaine dose and conditioning order. On day 3, mice were tested (Test 1) for 15 minutes, with parameters of chamber, floor, and time exactly as they were at baseline. Mice received an additional 4 conditioning trials across days 4-11, followed by 5 consecutive test sessions (extinction) across days 12-16. Conditioning and test parameters were identical to prior sessions.

THC Place Conditioning

A timeline of THC place conditioning is provided in (Figure 4). 320 mice were purchased from the Jackson Laboratory across 9 cohorts (5 edible, 4 injected) and arrived on PND 21±1 or 56±1 for adolescents or adults, respectively. Each cohort represented a different combination of THC dose and ROA. The first 5 cohorts received edible dough, and the order of THC dose was pseudorandomized (0.75, 3.0, 1.5, 0.0, and 6.0 mg/kg). The last 4 cohorts received injection, and the order of THC dose was pseudorandomized (0.75, 3.0, 1.5, 6.0 mg/kg), with mice receiving 0.0 mg/kg THC equally distributed among the 4 cohorts. Mice were pair-housed with an age-matched cage mate under a 12-hour, reverse light-dark cycle in a temperature- and humidity-controlled vivarium. Mice had ad libitum access to food and water, except when in conditioning chambers, and had ad libitum access to only water during edible dough access. Mice had an additional 2-4 days of habituation, which included receipt of an ear punch for designation, initiation of daily body weight measurement prior to dark cycle onset, initiation of daily dough access or daily injection, and a single 2-hour habituation session in the testing room, which was either preceded by control dough access (edible) or concurrent with vehicle administration (injected). A final habituation day included 30 minutes of habituation to the testing room followed by a 30-minute habituation session in the testing chambers, with floor textures covered by a single sheet of paper [Cunningham, 1999; Fritz, 2016], following control dough access or vehicle injection. Mice were given dough access immediately prior to being transported to the testing room or were given an injection immediately prior to placement in the chamber. These 30-minute (edible) and 0-minute (injected) pre-treatment times were used for the remainder of the study.

On PND 26 or 61 (experimental day 0), mice received control dough access or vehicle injection and underwent a baseline floor preference assessment, which was 30 minutes in duration and used both floor textures in a sided-by-side arrangement. Floor texture side was counterbalanced within age group. Prior to day 1, mice were rank-ordered by time spent on their

non-preferred floor texture within each age group. The 2 adult and 2 adolescent mice with the lowest times per cohort (and their cage mates) were excluded from the remainder of the study. Exceptions include assessing all mice receiving 0.0 mg/kg by injection as well as excluding mice (and their cage mates) which failed to consume 100% of control dough on day 0 in place of mice with low baseline times.

Days 1-6 consisted of 3 trials of THC place conditioning, with one session per day. Mice were placed in the conditioning chamber for 60 (edible) or 45 minutes (injected) on a single floor texture. THC was paired with the non-preferred floor texture, and conditioning order (THC vs control/vehicle) was counterbalanced within age and dose. Cage mates received the same THC dose and conditioning order. On day 7, mice were tested (Test 1) for 30 minutes, with parameters of chamber, floor, and time exactly as they were at baseline. Mice received an additional 2 weeks of place conditioning, days 8-13 (conditioning), day 14 (Test 2), days 15-20 (conditioning), and day 21 (Test 3). Conditioning and test parameters were identical to prior sessions.

Statistical Analysis

Statistical analyses were conducted using Excel (Microsoft, Redmond, WA), MATLAB (MathWorks, Natick, MA), Prism (GraphPad, San Diego, CA), and/or SPSS (IBM, Armonk, NY).

For cocaine place conditioning, variables of interest included body weight, locomotor activity, time spent on grid floor, and time spent on the non-preferred floor. Parametric analyses were conducted unless data were determined to violate the assumption of normality, using Shapiro-Wilk tests within each condition, in which case non-parametric analyses were used. Body weight (g) was analyzed at baseline and at test 6 using a 2-way ANOVA (Age x Dose) and across conditioning sessions using a 3-way mixed ANOVA (Session (repeated) x Age x Dose). Locomotor activity (total distance traveled (cm)) was analyzed at baseline using a 2-way ANOVA (Age x Dose), across tests using a 3-way mixed ANOVA (Test (repeated) x Age x Dose), for each test individually using 2-way ANOVA's (Age x Dose), and for each CS- (saline) and CS+ (cocaine) conditioning session using Kruskal-Wallis tests (Dose) within each age. For all cocaine place conditioning analyses, significant results were followed up using 1-way ANOVA, t-test, Dunn's test, Dunnett's test, and/or Tukey's HSD where appropriate. To

determine if mice had a floor texture preference, time spent on the grid floor (sec/min = (total time spent on grid floor / total time spent on either floor) * 60)) at baseline was analyzed using an independent samples t-test (Age) and using a single sample t-test (vs 30 sec/min) within each age. To determine if there were any initial differences, time spent on the non-preferred floor (sec/min) at baseline was analyzed using a 2-way ANOVA (Age x Dose) and using a single sample t-test (vs 30 sec/min) within each age. To determine the impact of cocaine place conditioning, change in time spent on the non-preferred floor (test – baseline, sec/min) was analyzed using a 3-way mixed ANOVA (Test (repeated) x Age x Dose), using 1-way ANOVA's (Dose) for each test within each age, and using single sample t-tests (vs 0 sec/min) for each combination of test, age, and dose.

For THC place conditioning, variables of interest included dough consumption, THC dose consumed, body weight, time spent on grid floor, time spent on the non-preferred floor. Parametric analyses were conducted unless data were determined to violate the assumption of normality, using Shapiro-Wilk tests within each condition, in which case non-parametric analyses were used. For mice in the edible condition, uneaten dough was weighed daily and used to calculate % of dough consumed and THC dose consumed. Amount of dough consumed (% consumed) was analyzed using a Kruskal-Wallis test (Dose) for each conditioning session for both control and THC dough separately for each age. For THC dough sessions, THC dose consumed (mg/kg) was analyzed using a Mann-Whitney test (Age) for each conditioning session within each dose provided. Body weight (g) was analyzed at baseline using a 3-way ANOVA (Age x Dose x ROA) and using 2-way ANOVA's (Age x Dose) within each ROA. In addition, the difference in body weight following edible THC and control dough consumption (THC – control) was calculated for each trial and averaged across conditioning trials for each week. The impact of THC on body weight differences was determined using 3-way mixed ANOVA's (Week (repeated) x Dose x Order) for each combination of age and ROA and using 2-way ANOVA's (Dose x Order) for each week for each combination of age and ROA.

Locomotor activity (total distance traveled (cm)) was analyzed separately for habituation, baseline, and test (1-3) sessions using 3-way ANOVA's (Age x Dose x ROA) and using 2-way ANOVA's (Age x Dose) within each ROA. For conditioning sessions, relative locomotor activity was determined by calculating distance traveled (cm) following THC administration as a percent of distance traveled (cm) following control/vehicle administration for each trial. These

values were then averaged across the 3 trials for each week to create weekly relative locomotor activity values (% of control/vehicle). The impact of THC on relative locomotor activity was analyzed using a 4-way mixed ANOVA (Week (repeated) x Age x Dose x ROA), 3-way mixed ANOVA's (Week (repeated) x Age x Dose) within each ROA, and 2-way ANOVA's (Age x Dose) for each combination of Week and ROA. For mice in the injected condition, the number of fecal boli were counted following trial 2b for all mice and following trials 1a and 3c in a subset of mice (0.0, 1.5, 6.0 mg/kg THC groups). The number of fecal boli counted following vehicle sessions was analyzed a using 4-way mixed ANOVA (Session (repeated) x Age x Dose x Order). To determine the impact of THC, the difference in fecal boli counted following THC vs vehicle sessions (THC – vehicle) was analyzed using a 2-way ANOVA (Age x Dose) for each trial separately.

To determine if mice had a floor texture preference, time spent on the grid floor (sec/min = (total time spent on grid floor / total time spent on either floor) * 60)) at baseline was analyzed using a 3-way ANOVA (Age x Dose x ROA), using a 2-way ANOVA (Age x Dose) within each ROA, and using a single sample t-test (vs 30 sec/min) for each combination of age and ROA. To determine if there were any initial differences, time spent on the non-preferred floor (sec/min) at baseline was analyzed using a 3-way ANOVA (Age x Dose x ROA), using a 2-way ANOVA (Age x Dose) within each ROA, and using a single sample t-test (vs 30 sec/min) for each combination of age and ROA. To determine the impact of THC place conditioning, change in time spent on the non-preferred floor (test – baseline, sec/min) was analyzed using a 4-way mixed ANOVA (Test (repeated) x Age x Dose x Order) within each ROA, using a 3-way mixed ANOVA (Test (repeated) x Dose x Order) for each age within each ROA, and using a 2-way ANOVA (Dose x Order) for each test separately for each combination of age and ROA. For these analyses, mice in the edible condition which were assigned to the 6.0 mg/kg dose or those which ever consumed 0% of dough, or which ever averaged < 50% dough consumption for any single week were excluded. Given the variability in THC dose consumed by mice in the edible condition at both the 3.0 and 6.0 mg/kg doses provided, additional analyses were run for these mice separately for 3.0 and 6.0 mg/kg doses within each age. Change in time spent on the non-preferred floor was analyzed using a 2-way ANOVA (Test x Dose). For 3.0 mg/kg, the factor of Dose was defined as mice consuming < 3.0 mg/kg vs 3.0 mg/kg, and for 6.0 mg/kg, defined using a median split as those consuming low vs high edible THC doses. In addition, Pearson

correlations were run using average THC dose consumed in weeks 1, 2 and 3 and change in time spent on the non-preferred floor on tests 1, 2, and 3. For all THC place conditioning analyses, significant results were followed up using 1-way ANOVA, t-test, Dunn's test, Dunnett's test, and/or Tukey's HSD where appropriate.

RESULTS

Cocaine: Body Weight

Adolescent mice weighed less and gained more weight than adult mice. Body weight was analyzed at baseline, on the final test (test 6), and across conditioning sessions. Compared to adult mice, adolescent mice weighed less at baseline, $F(1, 42) = 124.45$, $p < .001$ and at test 6, $F(1, 42) = 132.77$, $p < .001$ (Figure 5A); however, adolescent mice had a greater increase in weight from baseline to test 6, $F(1, 42) = 579.68$, $p < .001$ (Figure 5B). Across conditioning sessions, Mauchly's test indicated a violation of sphericity, $\chi^2(44) = 343.70$, $p < .001$, so Greenhouse-Geisser corrected degrees of freedom were used. There was a significant main effect of both Session, $F(2.05, 85.88) = 102.08$, $p < .001$, and Age, $F (1, 42) =$ on body weight, as well as a Session x Age interaction, $F(2.05, 85.88) = 51.80$, $p < .001$, also indicating lower overall weight and a greater change in weight in adolescent mice (Figure 5A). There were no main effects or interactions of Dose on any body weight measures, $F's < 2.07$, $p's > .145$.

Cocaine: Locomotor Activity

Locomotor activity was analyzed for baseline, test, and conditioning sessions (Figure 6). Adolescent mice were more active at baseline, and there were variations in activity across tests based on age, dose, and test. At baseline, there was a significant main effect of Age, $F(1, 42) = 35.75$, $p < .001$, with adolescent mice traveling a greater distance than adult mice, but no main effect of Dose or interaction, $F's < 0.73$, $p's > .488$. Across tests 1-6, Mauchly's test indicated a violation of sphericity, $\chi^2(14) = 78.53$, $p < .001$, so Greenhouse-Geisser corrected degrees of freedom were used. There was a significant main effect of both Test, $F(2.49, 104.64) = 17.36$, $p < .001$, and Dose, $F(2, 42) = 3.78$, $p = .031$, with Tukey's HSD indicating greater distance traveled by mice receiving 5 vs 1 mg/kg cocaine, $p = .034$, as well as a significant Test x Age interaction, $F(2.49, 104.64) = 3.20$, $p = .034$. No other main effects or interactions were significant, $F's < 2.31$, $p's > .135$. Considering the effect of Test and it's interaction with Age, tests 1-6 were analyzed separately. There was a significant main effect of Age in tests 2 and 4, $F's > 4.34$, $p's < .044$, with less distance traveled by adolescent vs adult mice. There was also a significant main effect of Dose in test 1, $F(2, 42) = 3.36$, $p = .044$, and a trend towards a main

effect of Dose in tests 2, 4, and 5, F's > 2.93, p's < .065, but Tukey's HSD indicated no significant differences between doses, p's > .050.

Cocaine produced robust locomotor stimulation on cocaine conditioning sessions in both ages and had some impact on activity levels on saline conditioning sessions. Locomotor activity was analyzed separately for each age on both saline (CS-) and cocaine (CS+) conditioning sessions (Figure 7). For CS- sessions, there was a significant effect of Dose in adolescent mice on session 2d, $\chi^2(2) = 8.44$, p = .015, with Dunn's test indicating that mice receiving either 1 or 5 mg/kg cocaine traveled a greater distance than mice receiving 0 mg/kg cocaine, p's < .039 (Figure 7A), and there was a trend towards a main effect of Dose in adult mice on sessions 2a and 2c, χ^2's > 4.84, p's < .090, with Dunn's test indicating that mice receiving 5 mg/kg cocaine traveled a greater distance than mice receiving 0 mg/kg cocaine, p's < .029 (Figure 7B). Looking across CS- sessions for mice receiving 0 mg/kg cocaine within each age, there was a significant main effect of session in adolescent mice, F(4, 28) = 4.94, p = .004, but not adult mice F(4, 28) = 1.08, p = .381.

For CS+ sessions, in adolescent mice, there was a significant main effect of Dose on sessions 2c and 2d, χ^2's > 8.38, p's < .016, and a trend towards a main effect of Dose on sessions 1a and 2b, χ^2's > 5.69, p's < .059, with Dunn's test indicating greater distance traveled by mice receiving 5 mg/kg cocaine than mice receiving either 0 or 1 mg/kg cocaine on all 4 sessions, p's < .049 (Figure 7C). In adult mice, there was a trend towards a main effect of Dose on session 2b, $\chi^2(2) = 5.99$, p = .050, and a significant main effect of Dose on all other sessions, χ^2's > 7.12, p's < .029. Dunn's test indicated that mice receiving 5 mg/kg cocaine traveled a greater distance than mice receiving 0 mg/kg cocaine on all sessions, p's < .021, and mice receiving 1 mg/kg cocaine on sessions 2c and 2d, p's < .004 (Figure 7D).

Cocaine: Baseline Floor Preference

At baseline, time spent on the grid floor did not differ between adolescent and adult mice, t(46) = 1.23, p = .224, and was not different from chance (30 sec/min) in either age, t's < 1.49, p's > .152 (Figure 8A). There was no significant main effect of Age or Dose, or interaction, on time spent on the non-preferred floor at baseline, F's < 0.39, p's > .534. However, time spent on the non-preferred floor was significantly different from chance (30 sec/min), t's > 7.20, p's < .001, and was thus statistically non-preferred (Figure 8B).

Cocaine: Place Conditioning

The change from baseline in time spent on the non-preferred floor following conditioning was analyzed for all tests (tests 1-6) (Figure 9). Compared to saline, cocaine produced a conditioned place preference in adult mice but not in adolescent mice. Across tests, there was a main effect of both Test, $F(5, 42) = 3.66$, $p = .003$, and Dose, $F(2, 42) = 4.24$, $p = .021$, with Tukey's HSD indicating a greater increase in time spent on the non-preferred floor at both tests 2 and 3 vs test 1, p's $< .049$, and for mice receiving 5 vs 0 mg/kg cocaine, $p = .016$ (Figure 9A). However, there was no significant main effect of Age and no significant interactions, F's < 1.91, p's $> .160$. Considering the effect of Test and an a priori decision to examine the results of place conditioning in each age, the effect of Dose on change in time spent on the non-preferred floor was further analyzed separately for each test within each age. In adolescent mice, there was a trend towards a main effect of Dose in test 6, $F(2, 21) = 2.67$, $p = .092$, but no significant effect of Dose on any other test, F's < 1.83, p's $> .184$ (Figure 9B). In adult mice, there was a significant main effect of Dose on test 2, $F(2, 21) = 6.32$, $p = .007$, a trend towards a main effect of Dose in tests 3-6, F's > 2.77, p's $< .086$, but no significant effect in test 1. Tukey's HSD indicated a greater increase in time spent on the non-preferred floor for mice receiving 5 vs 0 mg/kg cocaine in test 2, $p = .005$, and test 3, $p = .048$ (Figure 9C).

In contrast, when compared to neutral response (0 sec/min change in time spent on the non-preferred floor), cocaine produced a conditioned place preference in mice of both ages. Consistent with some cocaine place conditioning literature which analyzes a measure of time spent in the cocaine-paired context vs chance performance [Poltyrev, 2013; Zakharova, 2009a; Zakharova, 2009b], change in time spent on the non-preferred side was also compared to chance (0 sec/min) for each combination of age, dose, and test. In adolescent mice, time spent on the non-preferred floor differed significantly from chance in tests 1-3 and 5-6 in mice receiving 1 mg/kg cocaine, t's > 2.46, p's $< .044$, and in tests 2-5 in mice receiving 5 mg/kg cocaine, t's > 3.56, p's $> .010$ (Figure 9B). All other conditions in adolescent mice failed to reach significance, p's $> .054$. In adult mice, time spent on the non-preferred floor differed significantly from chance in tests 3-4 in mice receiving 1 mg/kg cocaine, t's > 2.89, p's $< .024$, and in tests 1-6 in mice receiving 5 mg/kg cocaine, t's > 3.02, p's $< .021$ (Figure 9C). All other conditions in adult mice failed to reach significance, p's $> .058$.

THC: Edible Dough Consumption

Overall, control dough was well consumed, with a slight decrease in consumption in some groups of adult mice. For mice in the edible condition, consumption of dough was analyzed as percent consumed for all control dough sessions (baseline, tests, and conditioning) and all THC dough sessions (conditioning), separately for each age (Figure 10). For control dough, there was no effect of Dose on percent consumed in any session in adolescent mice, χ^2's < 4.01, p's > .405 (Figure 10A), and for adult mice, there was a significant effect of Dose in conditioning session 1b, $\chi^2(4) = 12.41$, $p = .015$, with Dunn's test indicating reduced consumption of control dough in mice receiving 6.0 mg/kg THC vs all other doses, p's < .006, but no effect of Dose in any other sessions, χ^2's < 6.71, p's > .151 (Figure 10B).

THC produced a dose-dependent decrease in dough consumption, which was more pronounced in adult mice. For THC dough, there was a significant effect of Dose on percent consumed in all sessions (1b-3c), except the first (1a), for both adolescent, $\chi^2(4) > 21.40$, $p < .001$ (Figure 10D), and adult mice, $\chi^2(4) > 18.97$, $p < .002$ (Figure 10E). Dunn's test indicated reduced consumption of THC dough in mice receiving 6.0 mg/kg THC vs all other doses in sessions 1b-3c for both adolescent, p's < .005, and adult mice, p's < .016, as well as reduced consumption of THC dough in session 2c in adult mice receiving 3.0 mg/kg THC vs either 0.0 or 0.75 mg/kg THC, p's < .038. In addition, the actual THC dose consumed was compared directly between adolescent and adult mice for each THC dough session, separately for each THC dose provided (Figure 11). Adolescent mice consumed a greater THC dose when provided 6.0 mg/kg THC in session 1c, Mann-Whitney $U = 30.00$, n's = 12, $p = .014$, and session 2a, Mann-Whitney $U = 30.50$, n's = 12, $p = .014$. There was no effect of Age on THC dose consumed in any session for any other THC dose provided, Mann-Whitney U's < 48.00, n's = 12, p's > .177.

THC: Body Weight

Body weight was analyzed at baseline and across conditioning sessions (Figure 12). Adolescent mice and mice in the edible condition weighed less at baseline, and there was a non-systematic difference in body weight based on dose in some age/ROA conditions. At baseline, there was a significant main effect of Age, $F(1, 220) = 2728.17$, $p < .001$, Dose, $F(4, 220) = 3.33$, $p = .011$, and ROA, $F(1, 220) = 12.11$, $p = .001$, on body weight, as well as a significant Age x

Dose interaction, $F(4, 220) = 8.26$, $p < .001$, and a trend towards an Age x ROA interaction, $F(1, 220) = 3.78$, $p = .053$, but no other significant main effects or interactions, F's < 1.63, p's $> .169$. Considering the effect of ROA, and the addition of dough to the diet of mice in the edible condition, body weight was analyzed separately for each ROA. For mice in the edible condition, there was a significant effect of both Age, $F(1, 110) = 1348.00$, $p < .001$, and Dose, $F(4, 110) = 3.39$, $p = .012$, as well as a significant Age x Dose interaction, $F(4, 110) = 4.93$, $p = .001$. Looking at each age separately, in adolescent mice, there was a significant main effect of Dose, $F(4, 55) = 5.62$, $p = .001$, with Tukey's HSD indicating lower weight in mice receiving 0.0 mg/kg THC vs mice receiving either 0.75, 1.5, or 6.0 mg/kg THC, p's $< .023$, and in adult mice, there was a trend towards a main effect of Dose, $F(4, 55) = 2.08$, $p = .096$ (Figure 12B).

For mice in the injected condition, there was a significant main effect of Age, $F(1, 110) = 1381.86$, $p < .001$, as well as a significant Age x Dose interaction, $F(4, 110) = 4.96$, $p = .001$ Looking at each age separately, in adolescent mice, there was a main effect of Dose, $F(4, 55) = 2.62$, $p = .045$, with Tukey's HSD indicating lower weight in mice receiving 3.0 mg/kg THC vs mice receiving 6.0 mg/kg THC, $p = .033$, and in adult mice, there was also a significant main effect of Dose, $F(4, 55) = 3.83$, $p = .008$, with Tukey's HSD indicating higher weight in mice receiving 6.0 mg/kg THC vs mice receiving either 0.75, 1.5, or 3.0 mg/kg THC, p's $< .030$ (Figure 12D). Thus, there were generally differences in mouse body weight based on the cohort used (dose), regardless of age or ROA, prior to THC exposure.

THC produced an increase in body weight relative to control/vehicle for all combinations of age and ROA except for adult mice in the edible condition. Considering the differences in body weight between groups at baseline, the impact of THC on body weight across conditioning was analyzed using the difference between body weight the morning following a THC session and the morning following a control/vehicle session (THC – control/vehicle) within a given trial. Body weight differences were averaged across the 3 trials within each week, and average difference in body weight was analyzed across weeks, separately for each combination of age and ROA (Figure 13). In the edible condition, in adolescent mice, there was a trend towards a main effect of Dose, $F(4, 50) = 2.30$, $p = .072$, as well as a trend towards a Week x Dose x Order interaction, $F(8, 100) = 1.98$, $p = .056$ (Figure 13A), but no other significant effects, F's < 1.63, p's $> .201$. In adult mice, there was a significant main effect of Dose, $F(4, 50) = 3.20$, $p = .021$ (Figure 13B), but no other significant main effects or interactions, F's > 1.29, p's $> .260$.

In the injected condition, in adolescent mice, there was a significant main effect of both Week, $F(2, 100) = 7.38$, $p = .001$, and Dose, $F(4, 50) = 2.72$, $p = .040$, as well as significant Week x Dose, $F(8, 100) = 4.84$, $p < .010$, Week x Order, $F(2, 100) = 6.59$, $p = .002$, and Week x Dose x Order, $F(8, 100) = 2.44$, $p = .019$, interactions (Figure 13C). In adult mice, there was significant main effect of Dose, $F(4, 50) = 2.63$, $p = .045$, and a significant Week x Dose interaction, $F(4, 50) = 4.38$, $p < .001$, as well as a trend towards both a main effect of Week, $F(2, 50) = 2.96$, $p = .056$, and a Dose x Order interaction, $F(4, 50) = 2.18$, $p = .085$ (Figure 13D). There were no other significant main effects or interactions in either age, F's < 2.22, p's $< .142$.

Considering the interaction of Week with other factors in 3 of the 4 analyses, as well as the substantial growth of adolescent mice across weeks, average difference in body weight was further analyzed separately for each week. In the edible condition, in adolescent mice, there was a significant main effect of Dose in week 1, $F(4, 50) = 2.93$, $p = .030$, and a trend towards a Dose x Order interaction in week 2, $F(4, 50) = 2.44$, $p = .059$, and in adult mice, there was a trend towards a main effect of Dose in week 2, $F(4, 50) = 2.29$, $p = .072$, with Dunnett's test indicating a reduction in weight following THC for mice receiving 3.0 mg/kg THC vs mice receiving 0.0 mg/kg THC, $p = .034$ (Figure 13A). There were no other significant main effects or interactions in mice in the edible condition, F's < 2.64, p's $> .110$.

In the injected condition, in adolescent mice, there was a trend towards a main effect of Dose in week 1, $F(4, 50) = 2.22$, $p = .080$, a significant main effect of both Dose, $F(4, 50) = 6.34$, $p < .001$, and Order, $F(1, 50) = 9.37$, $p = .004$, in week 2, and a significant main effect of Dose, $F(4, 50) = 3.00$, $p = .027$, in week 3. Dunnett's test indicated an increase in weight following THC in week 2 for mice receiving 6.0 mg/kg THC vs mice receiving 0.0 mg/kg THC, $p = .004$ (Figure 13C). In adult mice, there was a significant main effect of Dose in both week 2, $F(4, 50) = 5.14$, $p = .002$, and week 3, $F(4, 50) = 3.95$, $p = .007$, as well as a trend towards a Dose x Order interaction in week 3, $F(4, 50) = 2.52$, $p = .053$. Dunnett's test indicated an increase in weight following THC in week 2 for mice receiving either 3.0 or 6.0 mg/kg THC vs mice receiving 0.0 mg/kg THC, p's $< .004$ (Figure 13D). There were no other significant main effects or interactions in mice in the injected condition, F's < 2.50, p's $> .111$.

THC: Locomotor Activity

Locomotor activity was analyzed for habituation, baseline, test, and conditioning sessions (Figure 14). In the absence of THC, adolescent mice in both ROAs were more active at baseline, and mice in the injected condition were consistently less active than mice in the edible condition. Otherwise, there was some variation in activity based on age and/or dose on tests sessions. There was a significant main effect of ROA at habituation, baseline, and all 3 tests, F's > 66.92, p's < .001. Therefore, these sessions were analyzed separately for each ROA. At habituation, in the edible condition, there was a significant main effect of both Age, $F(1, 110) = 7.89$, $p = .006$, and Dose, $F(4, 110) = 4.22$, $p = .003$, with greater distance traveled by adult mice, and less distance traveled by mice receiving control dough for 0.75 mg/kg THC vs either 1.5 or 6.0 mg/kg THC, p's < .019. In the injected condition, there was a trend towards a main effect of Age, $F(1, 110) = 2.83$, $p = .095$, and a significant main effect of Dose, $F(4, 110) = 3.88$, $p = .005$, with less distance traveled by mice receiving vehicle for 0.75 mg/kg THC vs either 0.0 or 6.0 mg/kg THC.

At baseline, there was a significant main effect of Age in both the edible, $F(1, 110) = 18.51$, $p < .001$, and the injected, $F(1, 110) = 85.50$, $p < .001$, conditions, with greater distance traveled by adolescent mice in both cases. In the edible condition, there was also a significant Age x Dose interaction, $F(4, 110) = 2.63$, $p = .038$, but no significant effect of Dose within either age, F's < 1.69, p's > .165.

At test 1, in the edible condition, there was a significant main effect of Dose, $F(4, 110) = 4.33$, $p = .003$, and a significant Age x Dose interaction, $F(4, 110) = 2.96$, $p = .023$. Looking within each age, there was a significant main effect of Dose in adolescent mice, $F(4, 55) = 5.04$, $p = .002$, with Tukey's HSD indicating greater distance traveled by mice receiving control dough for 0.0 mg/kg THC vs either 0.75 or 6.0 mg/kg THC and by mice receiving control dough for 1.5 mg/kg THC vs 0.75 mg/kg THC, p's < .032, as well as a trend towards a main effect of Dose in adult mice, $F(4, 55) = 2.43$, $p = .058$, with Tukey's HSD indicating greater distance traveled by mice receiving control dough for 6.0 mg/kg THC vs 0.75 mg/kg THC, $p = .036$. There were no significant main effects or interactions in mice in the injected condition, F's < 1.18, p's > .324. At test 2, in the edible condition, there was a significant main effect of Dose, $F(4, 110) = 3.55$, $p = .009$, and a significant Age x Dose interaction, $F(4, 110) = 4.07$, $p = .004$. Looking within each age, there was a significant main effect of Dose in adult, $F(4, 55) = 6.12$, $p < .001$, but not

adolescent, $F(4, 55) = 1.58$, $p = .194$, mice, with greater distance traveled in adult mice receiving control dough for 6.0 mg/kg THC vs all other doses, p's < .006. In the injected condition, there was a significant main effect of Dose, $F(4, 110) = 2.95$, $p = .023$, with Tukey's HSD indicating greater distance traveled by mice receiving vehicle for 3.0 mg/kg THC vs 1.5 mg/kg THC, $p = .019$, but no other significant main effects or interactions, F's < 1.09, p's > .344. Finally, at test 3, in the edible condition, there was a trend towards both a main effect of Dose, $F(4, 110) = 2.12$, $p = .083$, and an Age x Dose interaction, $F(4, 110) = 2.28$, $p = .065$. In the injected condition, there was a significant main effect of both Age, $F(1, 110) = 17.53$, $p < .001$, and Dose, $F(4, 110) = 5.05$, $p = .001$, as well as a trend towards an Age x Dose interaction, $F(4, 110) = 2.20$, $p = .073$. Looking within each age, there was a significant main effect of Dose in both adolescent, $F(4, 55) = 3.73$, $p = .009$, and adult, $F(4, 55) = 3.52$, $p = .013$, mice, with less distance traveled by mice receiving vehicle for 1.5 mg/kg THC vs 0.75 mg/kg THC in adolescent mice, $p = .013$, or vs 0.0 mg/kg THC in adult mice, $p = .007$.

Compared to control/vehicle, THC produced locomotor stimulation in adolescent mice in the edible condition and had differential effects based on age in the injected condition. For conditioning sessions, relative locomotor activity was determined by calculating distance traveled (cm) following THC administration as a percent of distance traveled (cm) following control/vehicle administration for each trial. These values were then averaged across the 3 trials for each week to create weekly relative locomotor activity values (% of control/vehicle). The impact of THC on relative locomotor activity was analyzed for each week, separately for each ROA (Figure 15).

In week 1, in the edible condition, there was a significant Age x Dose interaction, $F(4, 110) = 2.69$, $p = .035$ (Figure 15A), but no other significant main effects or interactions, F's < 1.91, p's > .113. Looking within each age, there was a significant main effect of Dose in adolescent, $F(4, 55) = 3.60$, $p = .011$, but not adult, $F(4, 55) = 0.27$, $p = .896$, mice, with Tukey's HSD indicating greater relative distance traveled by adolescent mice receiving 6.0 mg/kg THC vs 0.0 mg/kg THC. In the injected condition, there was a significant main effect of Age, $F(1, 55) = 9.54$, $p = .003$, with greater relative distance traveled by adolescent mice (Figure 15B), but no other significant main effects or interactions, F's < 1.20, p's > .314.

In week 2, in the edible condition, there was a significant main effect of Dose, $F(4, 110) = 3.82$, $p = .006$, and a significant Age x Dose interaction, $F(4, 110) = 2.84$, $p = .028$ (Figure

15A). Looking within each age, there was a significant main effect of Dose in adolescent, $F(4, 55) = 4.97$, $p = .002$, but not adult, $F(4, 55) = 0.72$, $p = .582$, mice, with Tukey's HSD indicating greater relative distance traveled by adolescent mice receiving either 3.0 or 6.0 mg/kg THC vs both 0.0 and 0.75 mg/kg THC, p's < .032. In the injected condition, there was a significant main effect of Age, $F(1, 110) = 6.36$, $p = .013$, and a significant Age x Dose interaction, $F(4, 110) = 3.37$, $p = .012$ (Figure 15B). Looking within each age, there was a significant main effect of Dose in adult, $F(4, 55) = 2.60$, $p = .046$, but not adolescent, $F(4, 55) = 1.71$, $p = 1.61$, mice.

Finally, in week 3, there was a significant Age x Dose interaction in the edible condition, $F(4, 110) = 2.48$, $p = .048$ (Figure 15A), but no main effect of Dose within either age, F's < 2.02, p's > .105, and no other significant main effects or interactions in either ROA, F's < 1.24, p's > .299.

THC: Fecal Boli

For mice in the injected condition, fecal boli produced during conditioning sessions were counted and analyzed for all mice on both sessions for trial 2b, and for a subset of mice on both sessions for trials 1a and 3c (Figure 16). Two mice were excluded for having a number of boli ≥ 3 SD beyond the mean of their respective groups on any given session (1 adult 3.0 mg/kg, 1 adult 6.0 mg/kg). Looking at just the vehicle sessions in the mice for which data was available for all 3 trials, there was a main effect of both Session, $F(2, 102) = 24.82$, $p < .001$, and Age, $F(1, 51) = 9.67$, $p = .003$, as well as a significant Session x Age interaction, $F(2, 102) = 6.10$, $p = .003$ (Figure 16A), but there were no significant main effects or interactions of the factors of Dose or Order, F's < 2.03, p's > .142. Looking within each session, there was a significant main effect of Age in session 1a, $t(61) = 4.98$, $p < .001$, and a trend towards a main effect of Age in session 3c, $t(61) = 1.73$, $p = .089$. The significant main effect of session was also present within each age, F's > 7.10, p's < .003. The difference between the number of fecal boli produced on THC and vehicle sessions (THC – vehicle) was used to examine the impact of THC on this measure, separately for each trial, using all mice for which a value was recorded on a given trial (Figure 16B). On trial 1a, there was a significant main effect of both Age, $F(1, 57) = 8.96$, $p = .004$, and Dose, $F(2, 57) = 44.64$, $p < .001$, as well as a trend towards an Age x Dose interaction, $F(2, 57) = 2.76$, $p = .072$. There was also a significant main effect of Dose in both trial 2b, $F(4, 116) = 29.52$, $p < .001$, and trial 3c, $F(2, 57) = 26.97$, $p < .001$. No other main effects or interactions

reached significance, F's < 2.62, p's < .111. In all 3 trials, and in both ages, Tukey's HSD indicated that all THC doses produced a decrease in the number of fecal boli compared to the 0.0 mg/kg THC dose, p's < .017, but no other significant differences between doses were detected, p's > .190.

THC: Baseline Floor Preference

Mice demonstrated a significant floor preference at baseline, which differed by ROA, but was consistent across ages within each ROA. At baseline, there was a significant main effect of ROA on time spent on the grid floor, $F(1, 220) = 33.78$, $p < .001$, as well as a trend towards a Dose x ROA interaction, $F(4, 220) = 2.32$, $p = .058$ (Figure 17). Looking at time spent on the grid floor within each ROA, there was no significant main effect of Age or Dose, or interaction, F's < 1.77, p's > .140. However, time spent on the grid floor differed significantly from chance (30 sec/min) for all combinations of age and ROA, t's > 2.08, p's < .042. Overall, mice of both ages in the edible condition had a preference for the grid floor, while mice of both ages in the injected condition had a preference for the hole floor. In addition, there was a significant main effect of ROA on time spent on the non-preferred floor, $F(1, 220) = 16.81$, $p < .001$, as well as a significant Age x ROA interaction, $F(1, 200) = 4.61$, $p = .033$. Looking at time spent on the non-preferred floor within each ROA, there was a significant main effect of Age in mice in the edible condition, $F(1, 110) = 6.84$, $p = .010$, with adolescent mice spending less time on their non-preferred floor than adult mice, but no other significant main effects or interactions for either ROA, F's < 1.94, p's > .108. However, time spent on the non-preferred floor differed significantly from chance (30 sec/min) for all combinations of age and ROA, t's > 10.76, p's < .001, and was thus statistically non-preferred.

THC: Edible THC Place Conditioning

The change from baseline in time spent on the non-preferred floor following conditioning was analyzed across all 3 tests, separately for each ROA (Figures 18-19). THC place conditioning produced neutral results in mice of both ages in the edible condition (Figure 18). Mice provided 6.0 mg/kg THC dough, as well as mice consuming 0% of THC dough on any occasion or averaging < 50% consumption of THC dough for any week, were excluded from these analyses (1 adolescent, 2 adult, 3.0 mg/kg THC dough provided). In the overall 4-way

mixed ANOVA, Mauchly's test indicated a violation of sphericity, $\chi^2(2) = 7.91$, $p = .019$, so Greenhouse-Geisser corrected degrees of freedom were used. There was a significant main effect of Test, $F(1.82, 140.15) = 3.81$, $p = .028$, and trend towards a main effect of Age, $F(1, 77) = 3.34$, $p = .072$, as well as a significant Test x Dose x Order interaction, $F(5.46, 140.15) = 2.29$, $p = .044$, and a trend towards a Test x Age interaction, $F(1.82, 140.15) = 2.49$, $p = .091$ (Figure 18A). Considering the significant interaction of Test in the 4-way mixed ANOVA, change in time spent on the non-preferred floor was further analyzed separately for each test using 3-way ANOVAs (Age x Dose x Order), which yielded no significant main effects or interactions at any test, F's < 1.57, p's > .219. Considering the trend towards interaction of Age in the 4-way mixed ANOVA, change in time spent on the non-preferred floor was further analyzed across tests within each age. In adolescent mice, there were no significant main effects or interactions, F's < 1.08, p's > .352 (Figure 18B). In adult mice, there was a significant Text x Dose interaction, $F(2, 28) = 3.89$, $p = .032$ (Figure 18C), but no other significant main effects or interactions, F's < 2.01, p's > .152. Finally, analysis of time spent on the non-preferred side was conducted separately for each test at each age as an a priori decision. In adolescent mice, there was a trend towards a main effect of Order in test 3, $F(1, 39) = 2.88$, $p = .098$, but no significant main effects or interactions for any test, F's < 0.96, p's > .367. In adult mice, there was a trend towards a main effect of Order in test 2, $F(1, 38) = 3.08$, $p = .088$, but no significant main effects or interactions for any test, F's < 2.13, p's > .112.

THC: Injected THC Place Conditioning

In the injected condition, THC place conditioning produced a conditioned place aversion at the highest dose, both overall and specifically in adult mice (Figure 19). The 4-way mixed ANOVA yielded a significant main effect of Dose, $F(4, 100) = 4.01$, $p = .005$, with Dunnett's test indicating a decrease in time spent on the non-preferred floor for mice receiving 6.0 mg/kg THC vs mice receiving 0.0 mg/kg THC, $p = .002$ (Figure 19A). In addition, there was a trend towards both a main effect of Test, $F(2, 200) = 2.82$, $p = .062$, and an Age x Dose x Order interaction, $F(4, 100) = 2.16$, $p = .080$. Considering the trend towards effect of Test in the 4-way mixed ANOVA, change in time spent on the non-preferred floor was further analyzed separately for each test using 3-way ANOVAs (Age x Dose x Order). There was a significant main effect of Dose in both test 1, $F(4, 100) = 3.44$, $p = .011$, and test 2, $F(4, 100) = 3.46$, $p = .011$ (Figure

19A), as well as a trend towards an effect of order in both test 1, $F(1, 100) = 2.98$, $p = .087$, and test 2, $F(1, 100) = 3.40$, $p = .068$. Compared to mice receiving 0.0 mg/kg THC, Dunnett's test indicated a decrease in time spent on the non-preferred floor for mice receiving 6.0 mg/kg in test 2, $p = .004$, and test 3, $p = .006$, and a trend towards a decrease for mice receiving 3.0 mg/kg in test 2, $p = .060$. There were no other significant main effects or interactions, F's < 1.95, p's $> .108$. Considering the trend towards interaction of Age in the 4-way mixed ANOVA, change in time spent on the non-preferred floor was further analyzed across tests within each age. In adolescent mice, there was a main effect of Dose, $F(4, 50) = 3.07$, $p = .025$ (Figure 19B), with a trend for a decrease in time spent on the non-preferred floor in mice receiving 6.0 mg/kg THC vs mice receiving 0.0 mg/kg THC, $p = .077$ (Dunnett), but no other main effects or interactions, F's < 1.75, p's $> .179$. In adult mice, there was a significant main effect of Dose, $F(4, 50) = 2.59$, $p = .048$, and a trend towards a Dose x Order interaction, $F(1, 50) = 2.30$, $p = .072$ (Figure 19C), but no other significant main effects or interactions, F's < 2.26, p's $> .138$. There was a significant decrease in time spent on the non-preferred floor for mice receiving 6.0 mg/kg THC vs mice receiving 0.0 mg/kg THC, $p = .029$ (Dunnett).

Finally, analysis of time spent on the non-preferred side was conducted separately for each test at each age as an a priori decision. In adolescent mice, there was a trend towards a main effect of Order in test 1, $F(1, 50) = 3.15$, $p = .082$, a trend towards a main effect of Dose in test 2, $F(4, 50) = 2.17$, $p = .086$, and a significant main effect of Dose in test 3, $F(4, 50) = 2.67$, $p = .043$ (Figure 19B), but no other significant main effects or interactions, F's < 2.06, p's $> .100$. Adolescent mice receiving 6.0 mg/kg THC had a decrease in time spent on the non-preferred floor vs mice receiving 0.0 mg/kg THC in test 2, $p = .032$ (Dunnett). In adult mice, there was a significant main effect of Dose, $F(4, 50) = 2.74$, $p = .039$, as well as a trend towards both a main effect of Order, $F(1, 50) = 3.43$, $p = .070$, and a Dose x Order interaction, $F(4, 50) = 2.33$, $p = .069$, in test 2, and a trend towards a main effect of Dose in test 3, $F(4, 50) = 2.12$, $p = .094$ (Figure 19C), but no other significant main effects or interactions, F's < 1.96, p's $> .114$. Adult mice receiving 6.0 mg/kg THC had a decrease in time spent on the non-preferred floor vs mice receiving 0.0 mg/kg THC in test 3, $p = .031$ (Dunnett).

THC: Edible THC Place Conditioning (THC Dose Consumed)

The results of THC place conditioning differed within each age based on the dose of edible THC consumed. Time spent on the non-preferred floor following conditioning was further analyzed in groups of mice displaying sufficient variability in edible THC dose consumed (3.0 and 6.0 mg/kg) using a 2-way ANOVA (Test x Dose) for each combination of age and dose provided (Figure 20). For mice provided 3.0 mg/kg THC, Dose was defined as consuming an average of 3.0 mg/kg vs < 3.0 mg/kg THC for a given week. In adolescent mice, there were no significant main effects or interactions, F's < 0.69, p's > .413 (Figure 20A). In adult mice, there was a significant main effect of Dose, $F(1, 30) = 5.11$, $p = .031$, with a decrease in time spent on the non-preferred floor in mice consuming the full 3.0 mg/kg THC dose provided (Figure 20B), but no other significant main effects or interactions, F's < 0.31, p's > .740. For mice provided 6.0 mg/kg THC, Dose was defined as high or low using a median split of average THC dose consumed each week within each age. In adolescent mice, there was a main effect of Dose, $F(1, 30) = 7.14$, $p = .012$, with Sidak's test indicating an increase in time spent on the non-preferred floor in adolescent mice consuming a high amount of THC in week 3, $p = .009$ (Figure 20C), but no other significant main effects or interactions, F's < 2.21, p's > 0.127. In adult mice, there were no significant main effects or interactions, F's < 0.82, p's > .452 (Figure 20D).

At the individual level, THC place conditioning outcomes were predictive of subsequent edible THC doses consumed within each age. To further leverage variability in edible THC dose consumed, Pearson correlations were run for all combinations of average THC dose consumed for each week and change in time spent on the non-preferred floor for each test, separately for each age and THC dose provided (Figure 21; Tables 1-4). In adolescent mice provided 3.0 mg/kg THC (Table 1), average THC dose consumed in week 3 was significantly positively associated with change in time spent on the non-preferred floor at both test 2, $r(10) = .59$, $p = .044$, and test 3, $r(10) = .61$, $p = .034$, and change in time spent on the non-preferred floor at test 2 was significantly positively associated with the same measure at both test 1, $r(10) = .60$, $p = .039$, and test 3, $r(10) = .94$, $p < .001$. In adolescent mice provided 6.0 mg/kg THC (Table 3), average THC dose consumed in week 3 was significantly positively associated with average THC dose consumed in week 2, $r(10) = .69$, $p = .013$, as well as change in time spent on the non-preferred floor at both test 1, $r(10) = .60$, $p = .039$ (Figure 21A), and test 3, $r(10) = .60$, $p = .038$.

In addition, change in time spent on the non-preferred floor at test 2 was significantly positively associated with the same measure at test 3, $r(10) = .67$, $p = .018$.

In adult mice provided 3.0 mg/kg THC (Table 2), average THC dose consumed was significantly positively associated in weeks 1 and 3, $r(10) = .88$, $p < .001$, and there was a significant negative association between average THC dose consumed in week 2 and change in time spent on the non-preferred floor at test 1, $r(10) = -.61$, $p = .036$ (Figure 21B), and between average THC dose consumed in week 3 and change in time spent on the non-preferred floor at both test 1, $r(10) = -.64$, $p = .026$, and test 2, $r(10) = -.62$, $p = .030$. In addition, change in time spent on the non-preferred floor was significantly positively associated for all tests, r's $> .61$, p's $< .034$. In adult mice provided 6.0 mg/kg THC (Table 4), average THC dose consumed was significantly positively associated in weeks 2 and 3, $r(10) = .65$, $p = .021$, and change in time spent on the non-preferred floor was significantly positively associated for all tests, r's $> .78$, p's $< .003$.

DISCUSSION

Summary

For cocaine place conditioning, adolescent mice weighed less but gained more weight than adult mice (Figure 5), as expected for this developmental period. However, cocaine had no impact on weight in either age. As expected, cocaine produced locomotor stimulation in mice of both ages (Figure 7). In addition, cocaine produced locomotor stimulation on some drug-free sessions (i.e. test and CS-) (Figures 6-7), suggesting conditioned locomotor stimulation to the drug-paired context. No initial preference for a particular floor texture was found in either age, indicating an unbiased conditioning apparatus, and in all conditions, cocaine was paired with a truly non-preferred texture (Figure 8). Overall, the highest dose of cocaine was effective in eliciting a conditioned place preference (Figure 9). However, it's effectiveness within each age was dependent on the analysis used, but a conditioned place preference was generally only present, or more robust, in adult mice (Figure 9).

For THC place conditioning, in the edible condition, control dough was very well consumed, but consumption of dough decreased with the addition of THC, especially at the highest dose provided (Figure 10). Over sessions, the THC dose consumed by mice given access to the highest dose of edible THC decreased significantly, and this decrease occurred more rapidly in adult mice (Figure 11). Adolescent mice weighed less but gained more weight than adult mice, as expected (Figure 12). However, weight differences were present at baseline based on dose and ROA, likely due to mice being ordered over several cohorts (Figure 12). THC administration impacted body weight for all combinations of age and ROA but was more pronounced and dose-dependent in mice in the injected condition (Figure 13). In addition, THC produced locomotor stimulation in adolescent mice consuming edible THC (Figure 15), contributed to age-related differences in locomotor activity in mice in the injected condition (Figure 15), and suppressed the excretion of fecal boli (Figure 16). Unlike mice used for cocaine place conditioning, mice used for THC place conditioning showed an initial preference for a particular floor texture, and the preferred texture differed by ROA (Figure 17). Thus, the apparatus used for THC place conditioning was biased. However, in all conditions THC was paired with a truly non-preferred texture (Figure 17). THC produced a neutral response in mice in the edible condition (Figure 18). However, in the injected condition, THC produced a

conditioned place aversion at the highest dose, which most pronounced in adult mice (Figure 19). For mice in the edible condition, the degree of reward/aversion produced by THC in individual mice was associated with the edible THC dose consumed in both ages (Figures 20-21; Tables 1-3), suggesting mice adjust their consumption of THC dough based on its subjective post-consumption hedonic properties.

Cocaine-Induced Effects on Locomotor Activity

Cocaine is a psychostimulant which reliably increases locomotor activity at low to moderate doses, but it can be without effect or decrease locomotor activity at high doses due to the induction of stereotypy [Tirelli, 2003]. However, adolescent rodents tend to be less sensitive to both the acute hyperlocomotive effects of cocaine and to psychostimulant-induced stereotypy than do adult rodents [Spear, 2000; Tirelli, 2003]. In the current study, cocaine administration at 5 mg/kg induced an increase in locomotor activity compared to saline administration in 4 of 5 conditioning sessions in adolescent mice and all 5 conditioning sessions in adult mice. Thus, the hyperlocomotive effect of cocaine was present at the highest dose for both ages, supporting hypothesis C2 (cocaine-induced hyperlocomotion in adult mice) but failing to support the locomotor component of hypothesis C3 (reduced sensitivity to cocaine in adolescent mice). In addition, cocaine resulted in an increase in locomotor activity across ages at test 1 and on a subset of saline conditioning sessions in both adolescent (session 2d) and adult (sessions 2a, 2c). One explanation for this hyperlocomotion on drug-free sessions is a conditioned locomotor effect of cocaine, consistent with those previously demonstrated in rats [Brown, 1992; Hotsenpiller, 2002]. However, in some instances, locomotor activity in saline-treated mice (0 mg/kg cocaine) decreased across sessions (i.e. adolescent CS-), while activity of cocaine-treated mice (1 or 5 mg/kg) remained consistent. Thus, an alternative explanation of cocaine's impact on locomotor activity on drug-free sessions is that it interfered with typical habituation processes taking place over repeated conditioning sessions.

Cocaine Place Conditioning

Mice were given a single day of habituation to the testing room for 2 hours, and the following day, adolescent (PND 26) and adult (PND 61) mice were assessed for baseline preference (30 mins) with both conditioning floor textures presented side-by-side. The results of

the baseline session indicated an unbiased apparatus with equal time spent on each floor texture across both ages and all doses, and cocaine was paired with the non-preferred floor for all mice. Cocaine induced an overall CPP at the 5 mg/kg dose across tests and ages. Looking within each age, compared to mice in the 0 mg/kg condition, cocaine induced a CPP at 5 mg/kg in adult mice at tests 2 and 3 but produced neutral results at all tests in adolescent mice, supporting hypothesis C1 (cocaine-induced CPP in adult mice) and the place conditioning component of hypothesis C3 (reduced sensitivity to cocaine in adolescent mice). On the contrary, when comparing the change in time spent on the non-preferred floor to chance performance (0 sec/min), cocaine induced a CPP in adult mice at 5 mg/kg (all tests) and 1 mg/kg (tests 3-4) and in adolescent mice at 5 mg/kg (tests 2-5) and 1 mg/kg (tests 1-3, 5-6). However, change in time spent on the non-preferred floor did not differ from chance performance for mice in the 0 mg/kg group at any test in either age. Thus, the interpretation that cocaine's capacity to induce CPP, and the degree to which this differs by age, is dependent upon the way in which the data are analyzed.

Several studies have examined cocaine place conditioning concurrently in adolescent and adult rodents in order to investigate age-related differences [Badanich, 2006; Balda, 2006; Brenhouse, 2008; Camarini, 2019; Campbell, 2000; Montagud-Romero, 2015; Montagud-Romero, 2017; Schramm-Sapyta, 2004; Zakharova, 2009a], and many claim age-related differences. However, close examination of details in a number of studies indicates that the literature overall contains methodological and statistical inconsistencies and limitations. For example, using B6 mice in a biased apparatus and pairing cocaine with the non-preferred floor across a range of doses, [Schramm-Sapyta, 2004] claimed similar CPP in mice of both ages. However, adolescent mice showed a much greater apparatus bias than adult mice at baseline ($p <$ .0001), and the change in time spent on the cocaine-paired context from baseline to test in saline treated mice differed in magnitude and direction, yet statistics were run just on time spent in the cocaine-paired context at test, ignoring initial differences. At least three studies make claims about age-related differences in cocaine's rewarding properties, yet fail to use saline control groups [Camarini, 2019; Montagud-Romero, 2015; Zakharova, 2019a]. In contrast, one study claimed to demonstrate similar rewarding capacity of cocaine across ages in mice, yet only used a single relatively-large dose (20 mg/kg), had no saline control, and analyzed a different duration of test session time for each age [Balda, 2006]. As a final example, using Swiss mice, [Camarini, 2019] claimed that pre-exposure to cocaine resulted in greater subsequent cocaine-

induced reward at 5 mg/kg in adolescent vs adult mice. However, in addition to not having a saline control group, only time in the cocaine-paired context at test was analyzed, yet the difference in this measure between adolescent and adult mice (~100 sec) was very similar to the difference between ages in time spent overall in the two conditioning contexts. Having used a 3-chamber apparatus, the missing time in conditioning contexts in adult mice was very likely spent in the center compartment, something which was statistically ignored. The above critique notwithstanding, there is mixed evidence overall for presence and direction of age-related difference in cocaine's rewarding properties. As a quality example, using Sprague-Dawley rats in early adolescence (PND 28+), late adolescence (PND 38+), or young adulthood (PND 53+) with cocaine administered at 0, 5, or 20 mg/kg and universally paired with each rats non-preferred context from baseline, [Badanich, 2006] found that while all ages showed cocaine-induced CPP at 20 mg/kg, only rats in early adolescence did so at 5 mg/kg.

As shown in the current study, drastic differences in the interpretation of cocaine place conditioning results can be obtained when analyzing the same dependent variable in comparison to a saline control group vs in comparison to hypothetical chance performance. Statistical and methodological inconsistencies in cocaine place conditioning studies comparing adolescent and adult rodents make an overall interpretation complicated. Using the most well-controlled analysis of the results obtained in the current study indicates that adult mice find cocaine rewarding at 5 mg/kg after five conditioning sessions, and that this effect lasts for just one extinction test session. However, neither adult mice conditioned with 1 mg/kg cocaine, nor adolescent mice conditioned with either cocaine dose demonstrate rewarding effects of cocaine. Collectively, the results obtained from cocaine place conditioning demonstrate cocaine-induced hyperlocomotion (as predicted) which did not differ by age (unpredicted), cocaine-induced conditioned locomotion which was similar in both ages, and cocaine-induced CPP (as predicted), the latter of which differed by dose and age (as predicted). This validation study confirmed the ability to detect age-related differences in place conditioning in our lab using a design with intermittent testing, and it set the stage for assessment of age-related differences in place conditioning with THC.

Edible THC Oral Self-Administration

Currently there are no publications available which simultaneously assess self-administration of THC in both adolescent and adult animals, likely making this study the first to do so. Self-administration of edible THC has previously been examined separately in adolescent and adult male mice using an escalating dose procedure [Smoker, 2019a; Smoker, 2019b]. Adult mice showed a substantial reduction in consumption at a 5 mg/kg THC dose provided, following their first exposure at this dose [Smoker, 2019b], while adolescent mice showed minimal reduction in consumption at a 6 mg/kg THC dose provided across 4 sessions [Smoker, 2019a], suggesting adolescent mice are willing to consume a greater dose of THC than adult mice. However, there were a number of methodological differences between the two studies precluding direct comparison, such as the doses used and the frequency of access.

The results of the current study indicate that adolescent mice are willing to self-administer edible THC to a greater degree than are adult mice. Consumption of THC dough did not differ from consumption of control dough in either age at 0.75 or 1.5 mg/kg provided, supporting hypothesis T1 (repeated consumption of edible THC). However, at the 6.0 mg/kg dose, consumption of THC dough was reduced in comparison to consumption of control dough (and all other THC doses) on the second access session and for the remainder of the study, supporting hypothesis T2 (dose-dependent reduction in consumption). The fact that nearly all mice (12/12 adolescent, 11/12 adult) consumed the entire serving of 6.0 mg/kg THC dough on the very first exposure indicates that the taste of the dough at this dose was likely not a factor in reducing consumption, but instead that post-ingestive effects of THC were responsible, similar to previously published data [Smoker, 2019b]. In addition, consumption of THC dough at the 3.0 mg/kg dose was reduced on a single session compared to control dough (and 0.75 mg/kg THC) in adult mice only, suggesting an age difference in sensitivity to the consumption-reducing effect of THC. Furthermore, direct comparison between ages of the actual dose of THC consumed revealed increased THC dose consumed for adolescent mice on sessions 1b and 1c when provided 6.0 mg/kg THC, supporting hypothesis T3 (higher self-administered THC dose in adolescent mice).

One possible factor contributing to the age difference in edible THC consumption apart from just the aversive component of its post-ingestive effects could be the motivation to consume the dough itself. If adolescent mice were more motivated to consume the dough, then they might

be willing to endure a more aversive outcome to do so. Inspection of control dough consumption for mice receiving the 0.0 mg/kg dose indicates that it was very well consumed by both ages, with 100% consumption on 126/126 and 125/126 occasions in adolescent and adult mice, respectively. Assessing adolescent and adult dough consumption in other procedures with an aversive consequence would help to clarify age differences in motivation to consume dough. However, this might be difficult, as adolescent rodents are generally less sensitive to aversive stimuli than adult rodents [Doremus-Fitzwater, 2016], for example, showing attenuated conditioned taste aversion following conditioning with lithium chloride [Clasen, 2016; Schramm-Sapyta, 2006].

THC-Induced Alterations in Body Weight, Locomotor Activity, and Fecal Boli

Given the distinct possibility that mice would demonstrate a neutral response (neither CPP nor CPA) to THC place conditioning, additional measures known to be impacted by THC were recorded and analyzed to verify the efficacy of administered THC, including body weight and locomotor activity. In addition, upon the observation that fecal boli produced by mice during conditioning with injected THC seemed to be bimodal (many or few) and to be associated with condition (vehicle or THC), an ad hoc decision was made to measure this variable on a subset of trials. Analysis of these measures also allowed comparison between adolescent and adult mice across several CB1R-mediated behaviors.

There were some differences in mouse body weight at baseline based on dose, except for adult mice in the edible condition. As mice had not yet been exposed to THC at this point, this effect is likely due to random factors inherent in ordering and receiving mice as separate cohorts (this limitation is discussed below). Therefore the impact of THC on body weight was analyzed within subjects by comparing change in weight following a THC conditioning day vs a control/vehicle conditioning day. Compared to control/vehicle, THC increased weight during the 24hrs following administration in all groups, except adult mice in the edible condition. This effect was very likely due to increased food intake, but this was not explicitly measured.

The appetite-stimulating effect of THC is well documented [Haney, 2007; NASEM, 2017; Nelson, 2018; Wenger, 2002; Wiley, 2005] and likely mediated by CB1R signaling in the hypothalamus [Wenger, 2002]. In contrast to the results of the current study, previous studies using adolescent rodents have found either a lack of effect of THC on body weight [Ellgren,

2007; Kasten, 2017], a decrease in both body weight and food intake [Scherma, 2016], or a change in direction of effect on food intake depending on procedure [Nelson, 2018]. Studies finding no effect used intermittent THC administration (every 3 days) and measured weight either every 3 days [Kasten, 2017] or as a change over the entire administration period [Ellgren, 2007]. Thus, the acute effects of THC on food intake were likely missed. The study demonstrating a decrease in weight and food intake used an escalating dosing procedure with twice daily administration in rats and found effects at higher doses (10-20 mg/kg/day) [Scherma, 2016]. THC has been shown to produce an inverted U-shaped dose-response effect on food intake, such that high doses actually produce a reduction [Wiley, 2005]. Therefore, it is not surprising the relatively high doses administered in rats, which are generally more sensitive than mice to the behavioral effects of a given dose of THC, produced this effect [Scherma, 2016].

The weight-increasing effect of THC in mice in the injected condition in the current study was very similar for both ages, but interestingly, didn't emerge until weeks 2 and 3 of conditioning. Possible explanations for this delayed emergence include tolerance to other effects of THC, which might have initially competed with food intake, or habituation to the conditioning procedure. In contrast, the effect of THC on weight in mice in the edible condition was only present in adolescent mice, and only in the first week. A probable explanation for these discrepancies is that mice of both ages significantly reduced their consumption of the highest dose of THC dough across weeks, but adolescent mice achieved a higher THC dose consumed than adult mice in week 1, permitting a THC-induced increase in food intake. Also of importance is that compared to other ROAs, oral administration of THC results in more variable absorption of THC and a greater relative level the active metabolite, 11-hydroxy-THC [Benjamin, 2016; Grotenhermen, 2003; Mantilla-Plata, 1975; Nadulski, 2005]. An acute increase in food intake following oral self-administration of THC has also been observed in adolescent rats consuming THC-laden cookies [Nelson, 2018]. Thus, age-related differences in THC metabolism or the relative impact of THC and 11-hydroxy-THC on food intake could also have contributed to differential effects of THC on body weight in between adolescent and adult mice in the edible condition. Taken together, the results of the current study indicate a dose-dependent increase in body weight that differs based on ROA, likely due to impartial dosing and/or THC metabolism in mice in the edible condition, and demonstrate the efficacy of administered THC at a dose as low as 3.0 mg/kg when injected.

Locomotor activity was measured during all sessions, habituation, baseline, conditioning, and tests. There was a pronounced effect of ROA on locomotor activity during all sessions, likely explained by an activity-reducing effect of handling/injection stress. During habituation, there was an effect of THC dose on locomotor activity across ages in both ROAs, prior to any THC exposure. In the edible condition, there was no discernable dose-related pattern to this effect, which might simply have been a product of mouse size, as the group-level pattern of activity levels in adolescent mice was similar to the group-level pattern of weight in this age, and adolescent (smaller) mice were less active than adult mice. In the injected conditioned, the effect could have been due to the amount of ethanol present in the vehicle, as mice of both ages in the 0.0 and 6.0 mg/kg groups, which had a vehicle with the highest (and matched) level of ethanol, had increased locomotor activity compared to mice in the 0.75 mg/kg group, which had a vehicle with the lowest level of ethanol. However, the difference in ethanol dose between these groups resulting from vehicle administration was only 0.040 g/kg (0.440 vs 0.400 g/kg), which should be well below the threshold to elicit a behavioral effect. In addition, a cohort effect is unlikely, as a subset of mice in the 0.0 mg/kg THC in the injected condition was run with every other group. Thus, the effect of THC dose in injected mice at baseline might have been random.

During the baseline session, adolescent mice were more active than adult mice in both ROAs, but there was no effect of THC dose. This is consistent with evidence that adolescent mice are more active in an inescapable novel environment [Laviola, 2003], as mice had been habituated to the chambers, but this was their first experience with the conditioning floor textures. The lack of effect of THC dose on activity during baseline (and during the first test) indicates that whatever might have been responsible for differences during habituation was no longer relevant after that session.

Locomotor activity on THC sessions was normalized to activity on control/vehicle sessions (% of control/vehicle) within mice and averaged for each week. In adult mice, THC did not impact locomotor activity, except for mice in the injected condition on week 2. This effect yielded an inverted U-shaped dose-response pattern overall, but individual THC doses did not differ statistically. In contrast to adult mice, THC produced effects in adolescent mice for both ROAs. In the edible condition, THC induced a dose-dependent increase in activity in both weeks 1 and 2. The same pattern of activity was present in week 3 but failed to reach statistical significance, likely due to adolescent mice reducing their consumption of THC at the highest

dose provided. In the injected condition, THC induced an age-dependent increase in relative activity in both weeks 1 and 2, with increased relative activity in adolescent vs adult mice. Inspection of the data suggests that this effect was driven by a non-significant dose-dependent increase in activity in adolescent mice which was absent in adult mice.

Administration of THC in rodents at moderate to high doses typically decreases locomotor activity [Lichtman, 2001; McMahon, 2007; Taffe, 2015]. However, when relatively low doses of THC are also administered, a biphasic or even triphasic dose-response pattern can be observed, with locomotor stimulation occurring at low doses [Katsidoni, 2013; Sanudo-Pena, 2000; Wiley, 2007]. The inverted U-shaped dose effect seen in adult mice in week 2 aligns well with this description. Additionally, the locomotor response to a given dose of THC has been shown to differ by age. Injected doses of THC which decrease activity in adult rodents have been shown to be without effect in adolescent rodents [Kasten, 2019; Schramm-Sapyta, 2007], but this is not always the case [Kasten, 2017]. The THC-induced increases in relative activity seen in adolescent mice in both ROAs could be explained by a shift in the dose-response curve in this age, such that THC doses which might produce a neutral response or hypolocomotion in adults fall on the ascending phase of the curve in adolescents, producing hyperlocomotion. Oral self-administration of THC in rats has been shown to produce hyperlocomotion in an open field in the first 10 minutes following a THC access period but not at 60 minutes following access, which the authors attributed to habituation to the novel aspect of the environment [Kruse, 2019]. In contrast, forced oral administration of THC in rats produced a relative difference in activity between low (1 mg/kg) and high (5 mg/kg) doses that did not differ across 3 separate 60-minute sessions [Dow-Edwards, 2008], suggesting a lack of effect of novelty. However, differences in administration, self- vs experimenter-administration, could have played a role is this discrepancy. Interestingly, the edible THC-induced hyperlocomotion in adolescent mice in this study is in opposition to edible THC-induced hypolocomotion in adolescent mice previously reported, using the same strain (B6), dose (6 mg/kg), and age (~PND 30) [Smoker, 2019a]. There were some procedural differences, escalating daily dose [Smoker, 2019a] vs static bi-daily dose (current study), but daily dosing would be more likely to induce tolerance to the descending phase (hypolocomotion) of THC's effect. Other factors that could have contributed to the difference in THC response seen in the two studies were the measurement of activity in the home cage vs in a conditioning chamber and the use of single- vs pair-housed arrangements.

Collectively, the results of the analysis of the impact of THC on locomotor activity overlap with the available literature in some ways, such as the biphasic pattern of response in adult mice in the injected condition and dose-dependent stimulation in adolescent mice in both ROAs. However, some results were unexpected based on previous literature. For example, there was an effect of THC on activity in the edible condition in adolescent mice which was absent in adult mice, yet adult mice would be expected to be more sensitive to THC. In addition, the effect of THC in adult mice in the injected condition in week 2 was not present in week 1, yet mice would be expected to be most impacted by THC initially and to develop tolerance thereafter. Regarding the results using edible THC, again, oral administration of THC yields a greater relative level the active metabolite, 11-hydroxy-THC, which compared to THC has a greater affinity for CB1Rs and is more potent in affecting locomotor activity in mice [Herkenham, 1990]. Assessment of the effect of age on THC's impact on locomotor activity had not previously been conducted using an oral ROA; therefore, a more thorough examination of this effect, including potential age-related differences in edible THC metabolism, would be valuable. Finally, very few studies have compared adolescents to adults in the place conditioning paradigm [Burgdorf, 2020; Quinn, 2008; Schramm-Sapyta, 2007], and only one has concurrently assessed locomotor activity, finding slight THC-induced hypolocomotion in rats of both ages [Schramm-Sapyta, 2007]. Thus, the novelty of many facets surrounding the assessment of locomotor activity in adults and adolescents during place conditioning in the current study, including floor textures, assignment to non-preferred floor, use of mice, and edible THC, are without precedent and complicate interpretation in the context of previous literature. Nevertheless, the results do demonstrate the efficacy of THC in affecting this behavior and demonstrate a differential impact based on age across both ROAs.

Measurement of fecal boli produced during THC place conditioning was conducted for a subset of mice in the injected condition, specifically all mice on trial 2b (the middle trial) and mice in the 1.5 and 6.0 mg/kg groups and concurrently-run mice in the 0.0 mg/kg group on additional trials 1a (first) and 3c (last). Analysis of fecal boli produced following vehicle administration in mice with data available for all trials 1a, 2b, and 3c indicated an increase in the number of boli produced by adolescent vs adult mice in all groups on trial 1a only (early adolescence), but no impact of THC dose on any trial. Compared to vehicle, THC induced a drastic reduction in fecal boli on all trials, in both ages, and at all doses. In addition, THC

produced a greater decrease in fecal boli in adolescent mice on trial 1a, which was likely due to a floor effect for THC in this measure (0 boli) combined with an elevated comparison value (vehicle session 1a) in adolescent mice. One explanation for the reduction in fecal boli following THC administration is delay of gastric emptying and depression of intestinal motility, which is well documented following cannabis or THC administration in humans and rodents [Anderson, 1975; Chesher, 1973; Grotenherman, 2003; NASEM, 2017], and due to activation of peripheral CB1Rs [Grotenherman, 2003; NASEM, 2017]. Another explanation for the reduction in fecal boli is an anxiolytic effect of THC during these sessions. Defecation in an open field was an early measure of emotionality in rodents [Walsh, 1976], and the number of fecal boli produced in a session is correlated with anxiety-like behavior across multiple assays of mouse anxiety [Milner, 2008]. Mice of both ages showed a reduction in fecal boli produced across vehicle sessions, consistent with attenuated emotional reaction to the conditioning context through habituation, and THC administration produced a substantial reduction in fecal boli compared to vehicle administration. Low doses of intraperitoneally-administered THC have been shown to be anxiolytic in both mice [Berrendero, 2002] and rats [Rubino, 2007]. Thus, the reduction in fecal boli seen following THC administration in the current study could have been due to reduced anxiety, depression of intestinal motility, or some combination of these two effects. Nevertheless, demonstration of this effect in the current study indicates that all injected doses, as low as 0.75 mg/kg, were biologically active.

THC Place Conditioning

All mice were given one day of habituation to the testing room (2 hrs) and one day of habituation to the conditioning chamber with floor textures covered (30 mins), both of which included a control dough access session (edible) or a vehicle injection (injected). Following these two days, adolescent (PND 26) and adult (PND 61) mice were assessed for baseline preference (30 mins) with both conditioning floor textures presented side-by-side. In contrast to cocaine place conditioning, mice used for THC place conditioning showed a group-level preference for one of the two floor textures, which differed based on ROA but was consistent between ages within each ROA. Thus, for THC place conditioning the apparatus was biased, with more mice preferring the grid floor in the edible condition and more mice preferring the hole floor in the injected condition. Using counterbalanced assignment of THC to floor type

would have created a situation where a larger proportion of mice in the edible condition with THC assigned to the hole floor (vs grid) would have been their non-preferred texture, and the opposite would have been the case for mice in the injected condition. The decision to assign THC to the non-preferred floor in all mice eliminated this difference between ROAs. As it was, mice in all combinations of ROA, age, and dose were assigned to a statistically non-preferred floor (< 30 sec/min at baseline), and the degree of non-preference did not differ by dose. However, time spent on the non-preferred floor was lower for adolescent mice than adult mice in the edible (but not injected) condition. This difference in time was due to greater variability in adolescent mice, but amounted to a difference of only 0.95 sec/min on average, likely negligible in the overall interpretation of the rewarding capacity of edible THC.

In the edible condition, THC place conditioning produced neither CPP nor CPA at any dose in either age, failing to support either hypothesis T4 (edible THC-induced CPP) or T5 (CPP in adolescent mice exclusively or at higher THC doses). A decision was made to exclude mice in the 6.0 mg/kg THC condition based on the high degree of variability in THC dose consumed, making group-wise interpretation difficult. However, exploratory analysis of the results of edible THC place conditioning either including mice in the 6.0 mg/kg group or excluding mice in both the 3.0 and 6.0 mg/kg groups also yielded null results. Therefore, under the experimental parameters used in this study, place conditioning with edible THC yields neutral results at the group level.

The variability in edible THC consumption at the 3.0 mg/kg and 6.0 mg/kg doses provided was leveraged to further examine the potential impact of THC on time spent on the non-preferred floor and yielded two interesting findings. First, across all three tests, adult mice which reduced their consumption from a full 3.0 mg/kg dose showed a relative CPP compared to mice which consumed the entire 3.0 mg/kg dose. Second, adolescent mice with the least decrease in consumption of the 6.0 mg/kg dose, thereby achieving higher doses, showed a relative CPP compared to mice with the greatest decrease in consumption at this dose, both across tests and specifically at test 3. The fact that grouping mice by their degree of edible THC consumption revealed differences in their behavioral display of its hedonic properties suggests that, at the individual level, mice might adjust their self-administered THC dose in order to maximize the associated reward and/or reduce the associated aversion. This idea is further supported by correlations between edible THC dose consumed and change in time spent on the

non-preferred floor. Indeed, in adolescent mice provided 6.0 mg/kg THC, not only was the THC dose consumed in week 3 significantly positively correlated with time spent on the non-preferred floor at test 3, it also shared this same association with time on the non-preferred floor at test 1 and just missed significance at test 2 ($p = .088$). Thus, the subjective hedonic value of edible THC in earlier tests predicted the degree to which adolescent mice were willing to consume it at a later time point, such that those mice which found it more rewarding (or less aversive) were those which subsequently self-administered a higher dose. Likewise, in adult mice provided 3.0 mg/kg THC, the dose consumed in both week 2 and week 3 was significantly correlated with the change in time spent on the non-preferred floor at all previous tests (i.e. test 1 with week 2, tests 1 and 2 with week 3). However, in contrast to adolescent mice, these correlations were all negative, such that mice which found edible THC more rewarding were those which subsequently self-administered lower doses. The claim that adult mice which found edible THC more rewarding were those which adjusted their consumption is based on the observation that even those mice which consumed the entire 3.0 mg/kg dose showed a change in time spent on the non-preferred floor comparable to (or slightly shifted towards reward) mice in the vehicle group. Thus, slightly reducing consumption (~30% reduction) might have allowed adult mice to titrate their self-administered THC dose to maximize its rewarding properties. In the other two conditions of interest, adolescent mice provided 3.0 mg/kg THC and adult mice provided 6.0 mg/kg THC, the dose-behavior relationships are not as compelling. In adolescent mice provided 3.0 mg/kg THC, the dose consumed in week 3 was significantly positively correlated with time spent on the non-preferred floor at both test 2 and test 3. However, this relationship appears to be driven by a single mouse. This mouse had a change in time spent on the non-preferred floor of 0.0, -12.0, and -11.0 sec/min in tests 1, 2, and 3, respectively, indicating a behavioral demonstration of aversion beginning at test 2. This mouse also consumed average THC doses of 3.0, 2.9, and 1.1 mg/kg in weeks 1, 2, and 3, respectively, indicating a drastic decrease in consumption in week 3. The combination of these two factors (the condition's most extreme decrease in both test time and dose consumed) in a single mouse produced the significant dose-behavior relationships, all of which were abolished with his exclusion (p's $> .414$). Finally, in adult mice provided 6.0 mg/kg THC, there were no instances of a significant relationship between dose consumed and change in time spent on the non-preferred floor. Collectively, while place conditioning with edible THC produced neutral results at the group level, grouped and

individual analyses based on dose consumed suggest that adolescent mice are less sensitive than adult mice to the hedonic effects of edible THC. Variability in THC dose consumed produced differences in the behavioral display of its hedonic properties at a higher dose provided in adolescent mice (6.0 mg/kg) vs adult mice (3.0 mg/kg). Furthermore these effects were produced by decreased consumption in adult mice (< 3.0 mg/kg) but by increased (relative) consumption in adolescent mice (6.0 mg/kg High). Specifically, adult mice showed a relative CPP when consuming an average of 2.2 mg/kg THC vs 3.0 mg/kg THC, while adolescent mice show a relative preference when consuming an average of 3.5-5.9 mg/kg THC vs an average of 1.1-4.6 mg/kg THC.

In contrast to edible THC, injected THC produced an overall CPA at the highest dose relative to mice receiving 0.0 mg/kg, supporting hypothesis T6 (injected THC-induced CPA). When looking within each age, THC impacted the change in time spent on the non-preferred floor for both adolescent and adult mice. However, following up the main effect seen in adolescent mice indicated that no THC dose differed significantly from 0.0 mg/kg. The only significant difference observed was between the 6.0 mg/kg and 0.0 mg/kg THC doses when a trend towards a main effect of dose in test 2 ($p = .086$) was followed by a Dunnett's post-hoc test, which is definitely a very liberal application of these statistical tests. Therefore, injected THC administered to adolescent mice produced neither CPP nor CPA. On the contrary, following up the main effect produced by THC in adult mice indicated that highest THC dose differed from 0.0 mg/kg, resulting in CPA. As with adolescent mice, following a trend towards a main effect of dose ($p = .092$) with a Dunnett's test at a particular time point (test 3) also yielded a significant difference between the 6.0 mg/kg and 0.0 mg/kg THC doses. Thus, there was greater support for CPA (vs vehicle) in adult mice. Taken together, only adult mice demonstrated a CPA, and only to the highest dose of THC, providing some support for hypothesis T7 (rightward shift in injected THC place conditioning results in adolescent mice), as adolescent mice were less sensitive the highest THC dose used, consistent with a rightward shift in their dose-response curve.

The combined results of place conditioning with edible THC and injected THC are in accord with previous studies in rodents demonstrating a reduced sensitivity to the hedonic properties of THC in adolescence [Quinn, 2008; Schramm-Sapyta, 2007]. Interestingly, although previous studies (and the current study) show reduced aversion to THC in adolescent

mice, the evidence for the ability of THC place conditioning to produce CPP in adolescent rodents is scant, if existing at all. Only one study has demonstrated a statistically significant THC-induced CPP in adolescent rodents. [Burgdorf, 2020] used adolescent female mice with a knock-in of a FAAH gene polymorphism and showed CPP to injected THC at 5 mg/kg. However, the genetic control group (lacking a FAAH polymorphism) actually showed a CPA to the same dose, and no vehicle control group was used for place conditioning, which is a severe limitation. Furthermore, this paper claims a demonstration of THC-induced CPP in male adolescent B6 mice (certainly worthy of full publication), but it is just briefly mentioned in the methods section (with some statistics), and all indications are that no vehicle control group was used [Burgdorf, 2020]. Thus, the hypothesis that edible THC would produce CPP (T4) which would be more likely in adolescent mice (T5) was optimistic at best and possibly quite presumptuous.

Results of place conditioning studies with THC are less consistent than the results from studies using many other drugs of abuse [Tanda, 2003; Tanda, 2016], and a number of experimental parameters have been shown to impact the results obtained using THC. Of course, the most critical parameter in THC place conditioning is the dose(s) used. In mice, all THC place conditioning has been conducted using i.p. injection, and THC-induced CPP has been demonstrated at 0.01, 0.03, 0.3, 1, and 5 mg/kg. With the exception of CPP at 0.03 and 0.3 mg/kg THC doses in a single study [Ponzoni, 2019], all other demonstrations of CPP in mice utilized THC pre-exposure [CPP 1 mg/kg; Ghozland, 2002; Soria, 2004; Valjent, 2000], nicotine pre-exposure [CPP 0.01 mg/kg; Ponzoni, 2019], or genetic manipulation (and no control group) [CPP 5 mg/kg; Burgdorf, 2020]. On the other hand, THC-induced CPA in mice has been demonstrated at 3, 5, 10, 15, and 20 mg/kg [Burgdorf, 2020; Cheng, 2004; Ghozland, 2002; Han, 2017; Hutcheson, 1998; Kardash, 2020; Soria, 2004; Valjent, 2000; Vann, 2008], with CPA to 5 mg/kg having been consistent across multiple studies. The outcome of THC place conditioning using oral administration, and specifically edible THC, was previously unknown. However, previous studies using edible THC self-administration in mice have indicated doses which produce behavioral alterations consistent with reward or aversion. In adult male mice, 2 mg/kg edible THC is fully consumed by all mice, while 5 mg/kg results in a decrease in consumption for some mice, but only following the initial exposure, and 10 mg/kg produces a profound reduction in consumption [Smoker, 2019b]. In adolescent male mice, 3 mg/kg edible THC is

very well consumed, while 6 mg/kg produces a decrease in consumption for some mice, and 12 mg/kg produces a profound reduction in consumption, especially following the initial exposure [Smoker, 2019a]. Thus, when providing edible THC to mice, 2-3 mg/kg is very well consumed, 5-6 mg/kg produces consumption for a subset of mice based on post-ingestive effects (not taste), and 10-12 mg/kg yields extremely poor consumption at the group level across repeated administrations [Smoker, 2019a; Smoker, 2019b]. Considering both the mouse place conditioning literature using injected THC and mouse self-administration of edible THC, the following doses were chosen for the current study for both ROAs, 0.0, 0.75, 1.5, 3.0, and 6.0 mg/kg. At the time of proposal [prior to Ponzoni, 2019], 1 mg/kg was the lowest THC dose to have been shown to induce CPP in mice (requiring THC pre-exposure), and 5 mg/kg had been shown to consistently induce CPA. Thus, the 0.75 and 6.0 mg/kg doses used in the current study encompassed these extremes for injected THC and included a dose (6.0 mg/kg) equal to and higher than doses known to produce an aversion-related behavior (CTA) in adolescent and adult mice, respectively, when self-administering edible THC. The intermediate 1.5 and 3.0 mg/kg THC doses provided a proportional increase (2x) across the range of doses used.

Outside of the conditioning dose used, one factor known to affect the outcome of THC place conditioning is pre-exposure to THC prior to the initiation of conditioning. [Valjent, 2000] demonstrated that pre-exposure to a single injection of 1 mg/kg THC in the home cage 24 hours prior to conditioning (thought to introduce mice to potential dysphoric effects of THC outside of the conditioning context) was sufficient to shift the result of conditioning with 1 mg/kg THC from neutral to CPP and of conditioning with 5 mg/kg THC from CPA to neutral. This pre-exposure procedure has subsequently been used to produce CPP to low-dose THC in other studies as a control for comparison using other manipulations [Ghozland, 2002; Soria, 2004]. Pre-exposure to THC outside of the conditioning context was not used in the current study. In the edible condition, pre-exposure would have been via injection or an initial edible THC access session. With pre-exposure via injection, mice would have experienced the effects of THC resulting from the pharmacokinetics of injected (i.p.) administration, which could have differed qualitatively from those subsequently resulting from oral (self-) administration, and the impact of this contrast on a mouse's subjective experience of THC's effects is unknown. With pre-exposure via edible THC, mice might have subsequently altered their consumption of edible THC at higher doses prior to the first conditioning session and not have experienced the effects

the initial full dose in the conditioning context. For these reasons, and to maintain as much consistency between edible THC and injected THC conditioning as possible, THC pre-exposure was not used. However, considering the effect of THC pre-exposure in other studies [Burgdorf, 2020; Ghozland, 2002; Soria, 2004; Valjent, 2000], its use in the current study might have revealed an absolute THC-induced CPP (vs control/vehicle), not just a relative CPP, and indicated differential sensitivity to this effect based on age.

Another factor contributing to THC place conditioning outcomes is the interval between consecutive THC doses. In rats, extending the interval between consecutive THC conditioning sessions from 2 to 4 days has been shown to produce a leftward shift in the dose response curve across a range of doses, such that CPP and CPA were produced by lower doses with an extended inter-dose interval [Lepore, 1995]. This is supported by a shift from a neutral to CPA outcome with extended inter-dose intervals in rats conditioned with 1 mg/kg THC [DeVuono, 2017]. These outcomes are likely related to extended inter-dose intervals reducing the development of tolerance and/or allowing for the completion or greater resolution of withdrawal-like effects. Furthermore, a number of THC place conditioning studies have used a rapid 1-day inter-dose interval (1 trial (2 injections) per day) [Burgdorf, 2020; Kardash, 2020; Le Foll, 2006; Ponzoni, 2019; Schramm-Sapyta, 2007]. While some studies justify this timing based on the relatively narrow developmental window of rodent adolescence [Burgdorf, 2020; Schramm-Sapyta, 2007], in other studies this timing is likely just used for convenience or out of convention based on other drugs [Kardash, 2020; Le Foll, 2006; Ponzoni, 2019]. In addition, in most studies employing 1-day inter-dose intervals, the daily vehicle conditioning session was conducted first, several hours prior to the THC session; however, in one study daily THC conditioning sessions were conducted just 7 hours prior to the vehicle conditioning sessions [Ponzoni, 2019]. In all cases, with just 24 hours between consecutive THC administrations, and several hours less than that between a THC and vehicle administration, carryover effects of THC are a valid concern [Tzschentke, 1998], especially given the ability of THC to produce spontaneous withdrawal-like effects at ~24 hours following repeated administration in adolescent and adult rodents [Murphy, 2017; Smoker, 2019a; Trexler, 2018]. The current study employed a 2-day inter-dose interval, with the exception of an extra day once per week due to testing. Extending this dosing interval would have greatly reduced the amount of conditioning trials that could have been conducted during the adolescent developmental window. Contracting this dosing interval would have made

carryover effects much more likely, especially when considering the extended duration of action of THC following oral administration in the edible condition [Grotenhermen, 2003; Hlozek, 2017]. However, it is possible that the dosing interval used in the current study was not ideal, especially given the novelty of combined edible THC self-administration and place conditioning, and that the results obtained could have differed substantially using alternate dosing intervals.

Several other variables, which are known to impact place conditioning with drugs of abuse, have varied in previous studies using THC but haven't received systematic investigation. These include pretreatment time, conditioning time, number of conditioning trials, CS pre-exposure, and animal housing (Cunningham, 1999; Kennedy, 2012; Tzschentke, 1998; Zakharova, 2009b). Regarding timing, THC pretreatment has nearly always been 0 minutes (immediate) in mice [longest = 5 minutes; Ponzoni, 2019], and it has most often been 10 minutes in rats [longest = 30 minutes; Mallet, 1998], with the discrepancy likely due to differences in metabolism between species or simply convention. THC conditioning times have ranged from 20-60 minutes in mice and 10-60 minutes in rats, with the majority of studies having used 30- or 45-minutes sessions. The current study used a 0-minute pretreatment time and 45-minute conditioning sessions for mice in the injected condition, both of which are the most commonly used times in mice. However, the use of edible (or simply oral) THC for place conditioning in the current study is without precedent. In the edible condition, the 30-minute pretreatment time (between dough access and conditioning) was chosen based on previous data in adult male mice that indicated the most pronounced hypothermic response at 60 minutes post-consumption (vs later time points), and the onset of a hypolocomotive response by 30 minutes or 60 minutes post-consumption for mice naïve to or experienced with edible THC consumption, respectively [Smoker, 2019b]. Furthermore, the 60-minute conditioning time is equal to the longest time previously used in rodents [Burgdorf, 2020; Zangen, 2006]. Although the effects of edible THC in mice have been shown to extend well beyond 60 minutes [Smoker, 2019a; Smoker, 2019b], this seemed like a reasonable cutoff to prevent a prolonged experience of social isolation and THC-induced aversive physiological effects like thirst and hypothermia [Fogel, 2017; Ginsburg, 2014; Kruse, 2019; Lichtman, 2001; Lile, 2013; Smoker, 2019a; Smoker, 2019b; Taffe, 2015]. Regarding the number of conditioning trials, THC place conditioning studies have invariably used 3-5 conditioning trials, with the exception of a single study in rats which used 8 trials (2 rounds of 4 trials) [Quinn, 2008]. Therefore, the 3 conditioning trials conducted per week/test in

current study is in line with previous research. However, the use of intermittent testing had only been employed once, but results indicated stable results across 2 tests and a protracted retest, suggesting no interference from tests conducted between conditioning rounds [Quinn, 2008]. In contrast to those results, place conditioning outcomes in the current study differed between early and later tests, which could have been due to discrepancies in trials per test (3 vs 4) and/or species used (mouse vs rat). In the context of place conditioning, CS pre-exposure would be exposure to the conditioning stimuli (e.g. floor textures) prior to conditioning with drug/vehicle, as is the case with baseline (pre-test) assessment. The use of a baseline session in the current study, which included both floor textures, is consistent with an overwhelming majority of THC place conditioning studies (23/25) conducting a pre-test assessment. Finally, although a few studies have failed to indicate animal housing arrangements [Burgdorf, 2020; Cheng, 2004; Han, 2017; Schramm-Sapyta, 2007], the use of pair-housed mice in the current study is in accord with the use of group-housed mice in all studies detailing this parameter. In summary, with respect to the potentially impactful variables outlined here, the parameters used in current study align with those used most frequently in mouse THC place conditioning studies, or when concerning the novel use of edible THC, were chosen based on the most relevant data available.

Limitations

The current study has limitations related to both the experimental procedures and generalizability of results. With respect to the experimental procedures, the most pronounced limitations are the use of multiple cohorts, the duration of dough access, and the use of ethanol in solutions. An ideal experimental design would be to have all conditions represented for each cohort assessed. For the current study, that would have required 80 mice per cohort (ROA x Age x Dose x Order x Cage mate; 2 x 2 x 5 x 2 x 2), and with 8 conditioning chambers available, running 10 squads per day. In addition, it would have required creating 5 unique edible doughs and 5 unique injection solutions per day. The combination of these factors is unattainable for any single human. Instead, a decision was made to execute the study with a strategy that would be manageable over several months of nearly-continuous testing, with a focus on precision and consistency with respect to all aspects of drug preparation, drug administration, mouse handling, and experimental timing and procedures. Mice in the edible condition were run over 5 cohorts, each representing a single THC dose (0.0-6.0 mg/kg). Mice in the injected condition were run

over 4 cohorts, each representing a single THC dose (0.75-6.0 mg/kg) plus 4 mice per age receiving the 0.0 mg/kg dose. The difference in number of cohorts between ROAs was due simply to the amount of time available each day, as study execution during the edible condition required more time for drug preparation, drug administration, and conditioning, which necessitated running fewer squads per day and more cohorts overall. Furthermore, running a subset of mice receiving the 0.0 mg/kg dose with each other dose in the edible condition (analogous to the injected condition) would have created at least one cohort containing a subset of mice from many or all of the dosing groups (0.0-6.0 mg/kg). This elicited concerns about the ability to make multiple edible THC preparations on a daily basis over multiple weeks without any degree of cross-contamination, loss in dose accuracy, difficulty in dissolving, or experimenter error. Keeping this cohort-based study execution in mind, inspection of data prior to conditioning could be informative. There were differences in weight based on dose (cohort) at baseline for all combinations of age and ROA, except adult mice in the edible condition. However, these differences were not consistent between ages within each cohort, suggesting they were not due exclusively to cohort-unique experiences, or that cohort-unique experiences differentially affected mice at different ages. On the contrary, there were no differences based on dose (cohort) at baseline in control dough consumption, locomotor activity, or in time spent on the non-preferred floor, all of which are more relevant to the hypotheses tested in this study. Thus, any effects resulting from the use of non-representative cohorts likely did not impact the interpretation of critical outcomes related to THC's hedonic properties.

Another limitation related to experimental procedures is the use of a 30-minute access session for edible dough consumption. This access time was chosen based on previous work and to ensure mice had sufficient time to consume the entire THC dose provided. However, on some occasions mice were observed to consume their dough portion in just a few minutes, while on other occasions mice took nearly the entire 30 minutes to consume their dough portion. The latency to consume dough was not recorded during this study, and so the 30-minute pretreatment time between the dough access session and the start of conditioning was probably realistically a range of approximately 35-55 minutes. The speed at which THC dough was consumed could have impacted the resulting THC pharmacokinetics and the relative time at which the onset of effects of THC were experienced with respect to placement in the conditioning chamber. The alternative approach of placing mice in the chamber exactly 30 minutes following consumption

would difficult to implement in practice and would not negate issues relating to THC pharmacokinetics. Essentially, this is a limitation inherent to combining self-administration and place conditioning.

A final noteworthy procedural limitation of the current study is the use of solutions containing ethanol. Due to its lipophilicity, THC solutions incorporate various solubilizing agents, and the variety and degree of use of these agents varies greatly from lab to lab. Importantly, the vehicle used for THC administration can influence its pharmacokinetics and the resulting effects on behavior [Tanda, 2016]. In addition, THC is generally stored as an ethanol suspension, and while some labs take measures to remove ethanol from THC vehicles via evaporation techniques, many do not, and many include additional ethanol as a component of the vehicle itself. As an illustration with just the THC place conditioning studies using B6 mice or mice bred on a B6 background, injection vehicles include Tween-80 : saline (1:19) [Burgdorf, 2020], ethanol : Cremophor EL : saline (1:1:8) [Cheng, 2004], and ethanol : Cremophor EL : distilled water (1:1:18) [Ghozland, 2002], and all solutions were made from THC-ethanol suspensions without evaporation. Ethanol is a rewarding drug and is generally able to induce CPP in mice in doses ranging from 1.5 to 4 g/kg, but this effect is dependent on many factors, such as strain, timing, age, and stress [Cunningham, 1992; Cunningham, 1993; Cunningham, 2003; Green, 2008; Silva, 2017; Song, 2007; Tzschentke, 1998]. Ethanol concentrations in the solutions used in the current study resulted in ethanol doses ranging from 0.006 to 0.045 g/kg in the edible condition and 0.400 to 0.440 g/kg in the injected condition. Ethanol doses administered in the edible condition were well below any known to produce CPP in mice. Ethanol doses administered in the injected condition were still below those known to produce CPP in mice and similar to those administered in other THC place conditioning studies in B6 mice [Cheng, 2004; Ghozland, 2002]. Considering the ethanol doses administered, the fact that mice received equal doses of ethanol on all conditioning and test sessions, and the fact that B6 mice are generally resistant to ethanol-induced CPP [Green, 2008], ethanol itself was likely not an important factor in the results obtained. However, given the evidence for a role of CB1R signaling in ethanol-related behaviors in rodents [Henderson-Redmond, 2016; Houchi, 2005; Hungund, 2003; Thanos, 2005; Marcus, 2017], and specifically its role in ethanol place conditioning [Houchi, 2005; Thanos, 2005], the potential for synergistic effects of combined ethanol-THC administration on subjective hedonic experience is an important consideration in

all THC place conditioning studies using ethanol-containing vehicles. There are currently no data available regarding the outcome of place conditioning using combined vs isolated administration of ethanol and THC, but studies of this nature would certainly be valuable.

With respect to the generalizability of results obtained in the current study, two factors stand out as most relevant, the sex and strain of mice used. Male mice were used for theoretical and practical reasons. Theoretically, in humans, males have higher rates of overall cannabis use and recent cannabis use, as well as higher rates of cannabis dependence in adolescence [Le Strat, 2009; Lopez-Quintero, 2011; Olfson, 2018]. Males also report increased positive subject effects of cannabis compared to females, and being male is associated with problematic cannabis use and a more rapid transition from use to problematic use [NASEM, 2017; Zeiger, 2010]. Practically, of the 24 rodent place conditioning studies currently available, the overwhelming majority of results are based on male rodents, as 19 studies used males exclusively, 2 included both sexes, 1 used females exclusively, and 2 failed to report sex. In addition, at the time of proposal of the current study, adult female mice had shown much less consistency in consumption of edible control dough [Smoker, 2019b], and assessment of consumption of edible THC dough in adolescent female mice had not been conducted. Thus, although interesting sex differences have been shown with respect to THC reward- and reinforcement-related behaviors and associated brain substrates [Burgdorf, 2020; Kruse, 2019], the current study applied the available resources to maximize the ability to assess the impact of age, dose, and ROA on THC's hedonic capacity. Also of practical consideration is the use of a single strain of mouse. The choice to use B6 mice was based on a number of factors, including their general popularity in biomedical research [Bryant, 2011] and previous demonstration of both CPP and CPA in this strain [Burgdorf, 2020; Cheng, 2004; Ghozland, 2002]. In addition, published studies of oral THC self-administration in mice have been conducted using the B6 strain exclusively [Leung, 2019; Smoker, 2019a; Smoker, 2019b]. However, it is to be acknowledged that the results of the current study might not be generalizable to other strains of mice or to rats.

Future Directions

In the U.S., approximately 50% of individuals use cannabis by the age of 20, yet the prevalence of daily use peaks before age 25 at only 10% of the population [Schulenberg, 2017]. Furthermore, of the individuals who identify as cannabis users, less than 1 in 5 would qualify as

having a cannabis use disorder [NASEM, 2017]. Cannabis use in adolescence is a risk factor for both cannabis use disorder and cannabis dependence [Clark, 1998; Forman-Hoffman, 2017]. However, research indicates that eventual problematic cannabis use isn't simply just an outcome of early cannabis use, but that the subjective experience associated with this early use might play an important role. Endorsing positive reactions to initial cannabis use in adolescence is dose-dependently (# of positive effects) predictive of subsequent cannabis dependence, even when controlling for a multitude of dependence-related variables, including both age at first cannabis use [Le Strat, 2009] and frequency of adolescent cannabis use [Fergusson, 2003]. The most novel finding in the current study was that individual differences in edible THC consumption were associated with a behavioral demonstration of its subjective hedonic properties. Interestingly, in adolescent mice, having an initial positive reaction (change in time on the non-preferred floor at test 1) to consumption of the highest dose of edible THC provided was predictive of subsequent elevated THC self-administration (edible THC dose consumed in week 3). These results align nicely with the relationship found in adolescent humans, and suggest that higher consumption of edible THC by adolescent mice across several repeated access sessions could serve as a model for problematic cannabis use. While the vast majority of THC self-administration studies have focused on the self-administration itself, or its pharmacological manipulation, recent studies have begun to examine behavioral and physiological consequences of self-administration [Freels, 2020; Kruse, 2019; Smoker, 2019a; Spencer, 2018]. Using an edible THC dose that produces a wide range of variability in consumption (e.g. 6 mg/kg in the current study), assessment could go the other direction and look for predictors of persistently elevated edible THC consumption among individual mice. The ability to predict which mice would likely show elevated THC self-administration would allow assessment of the effectiveness of implementing potential interventions prior to the initiation of THC use. While this is a broad direction in which to take this research, any contribution made to the understanding of effective interventions targeting development of problematic cannabis use would be quite valuable, especially considering the degree of investment needed for comparable longitudinal studies in humans.

Another, more concrete, direction in which to take this research would be using pharmacological manipulations to better understand the rewarding and aversive capacity of edible THC. Given the wealth of evidence implicating mu- and kappa-opioid receptor

functioning in the rewarding and aversive properties of THC, respectively [Ahmad, 2017; Berrendero, 2002; Cheng, 2004; Ghozland, 2002; Haney, 2015; Justinova, 2004; Kano, 2009; Maldonado, 2006; Onaivi, 1990; Spano, 2010; Tanda, 1997; Tanda, 2003], a combination of receptor subtype antagonism and edible THC self-administration could be used to support the claim that degree of edible THC consumption is related to its hedonic properties. For example, a mixed design (n x 2 x 2) across a number of days (n) of edible dough consumption (THC or control) with antagonist pretreatment (antagonist or vehicle) could be used. Providing mice with an edible THC dose which produces a wide range in degree of consumption, kappa-opioid receptor antagonism would be expected to shift the majority of mice to a relatively high THC dose consumed (by blocking aversive effects), mu-opioid receptor antagonism would be expected to shift the majority of mice to a relatively low THC dose consumed (by blocking rewarding effects), and vehicle administration would be expected to yield a wide degree of variability in THC dose consumed (based on individual subjective experience). Comparison groups of mice consuming control (instead of THC) dough would need to be included for a well-rounded design and because there is mixed evidence for the impact of opioid receptor activity on consumption of sweetened solutions [Ruegg, 1997; Sakamoto, 2015].

Finally, the edible THC model itself could be advantageous for use in addressing a number of other research questions. For example, edible THC could be used to examine the effects of prenatal THC exposure on both maternal care and offspring development, and could do so without using forced injection, gavage, or vapor inhalation in pregnant mice. Additionally, edible THC could be used as a relatively low-stress method of administration to examine the impact of developmental THC exposure on the interaction of eCB and hypothalamic–pituitary–adrenal (HPA) axis systems [Lee, 2012]. As oversight of the constituents of THC-based edible products and guidelines for their safe use is lagging behind the production, marketing, and state-level legalization of these products, cases of individuals experiencing extreme adverse reactions following their use are becoming more common [Benjamin, 2016; Bui, 2005; Favrat, 2005; Monte, 2015]. Edible THC for mice could be used to model a single high-dose exposure to THC, analogous to that resulting from misguided or unknowing administration of extremely high doses in humans, and to examine the resulting consequences. When provided a high concentration of THC in dough following multiple days of access to control dough, mice have been observed to consume up to 66 mg/kg in a single access session, resulting in a lack of

response to handling and lying prone and motionless for an extend period of time (Smoker – unpublished observations).

Conclusion

The overall theme to emerge from the studies conducted for this dissertation is a differential response between adolescent and adult mice to the hedonic properties of drugs of abuse. Specifically, adolescent mice appear to be less sensitive to rewarding properties of low doses of cocaine and to the rewarding and aversive properties of THC regardless of route of administration. Initiation of cannabis use during adolescence is a risk factor for a number of negative outcomes, including problematic cannabis use later in life, yet this specific outcome only develops in a subset of cannabis users. With a novel combination of edible THC self-administration and place conditioning, it was shown that the subset of adolescent mice which finds THC relatively rewarding also tends to self-administer more THC. A valuable application of these findings would be aiding in the development of strategies or markers to identify individuals at risk for problematic cannabis use prior to their reaching this negative endpoint.

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APPENDIX: TABLES AND FIGURES

Table 1. Average Weekly eTHC Dose Consumed & Change in Time Spent on the Non-Preferred Floor (Adolescent – 3.0 mg/kg)

	Dose Week 1	Dose Week 2	Dose Week 3	Δ Time Test 1	Δ Time Test 2	Δ Time Test 3
Dose Week 1	1					
Dose Week 2	.130	1				
Dose Week 3	-.158	.148	1			
Δ Time Test 1	.095	.186	.128	1		
Δ Time Test 2	-.204	-.092	.588*	.601*	1	
Δ Time Test 3	-.271	-.195	.612*	.459	.938**	1

* $p < .05$, ** $p < .01$ (2-tailed), N = 12

Table 2. Average Weekly eTHC Dose Consumed & Change in Time Spent on the Non-Preferred Floor (Adult – 3.0 mg/kg)

	Dose Week 1	Dose Week 2	Dose Week 3	Δ Time Test 1	Δ Time Test 2	Δ Time Test 3
Dose Week 1	1					
Dose Week 2	-.109	1				
Dose Week 3	.879**	.030	1			
Δ Time Test 1	-.434	-.608*	-.638*	1		
Δ Time Test 2	-.492	-.353	-.624*	.664*	1	
Δ Time Test 3	.111	-.474	-.190	.620*	.617*	1

* $p < .05$, ** $p < .01$ (2-tailed), N = 12

Table 3. Average Weekly eTHC Dose Consumed & Change in Time Spent on the Non-Preferred Floor (Adolescent – 6.0 mg/kg)

	Dose Week 1	Dose Week 2	Dose Week 3	Δ Time Test 1	Δ Time Test 2	Δ Time Test 3
Dose Week 1	1					
Dose Week 2	.427	1				
Dose Week 3	-.248	.693*	1			
Δ Time Test 1	.087	.462	.600*	1		
Δ Time Test 2	-.352	.238	.513	.527	1	
Δ Time Test 3	-.528	.251	.604*	.548	.667*	1

* $p < .05$ (2-tailed), N = 12

Table 4. Average Weekly eTHC Dose Consumed & Change in Time Spent on the Non-Preferred Floor (Adult – 6.0 mg/kg)

	Dose Week 1	Dose Week 2	Dose Week 3	Δ Time Test 1	Δ Time Test 2	Δ Time Test 3
Dose Week 1	1					
Dose Week 2	.293	1				
Dose Week 3	.227	.653*	1			
Δ Time Test 1	.253	.223	-.175	1		
Δ Time Test 2	.155	-.120	-.251	.787**	1	
Δ Time Test 3	-.041	-.076	-.331	.845**	.824**	1

* $p < .05$, ** $p < .01$ (2-tailed), N = 12

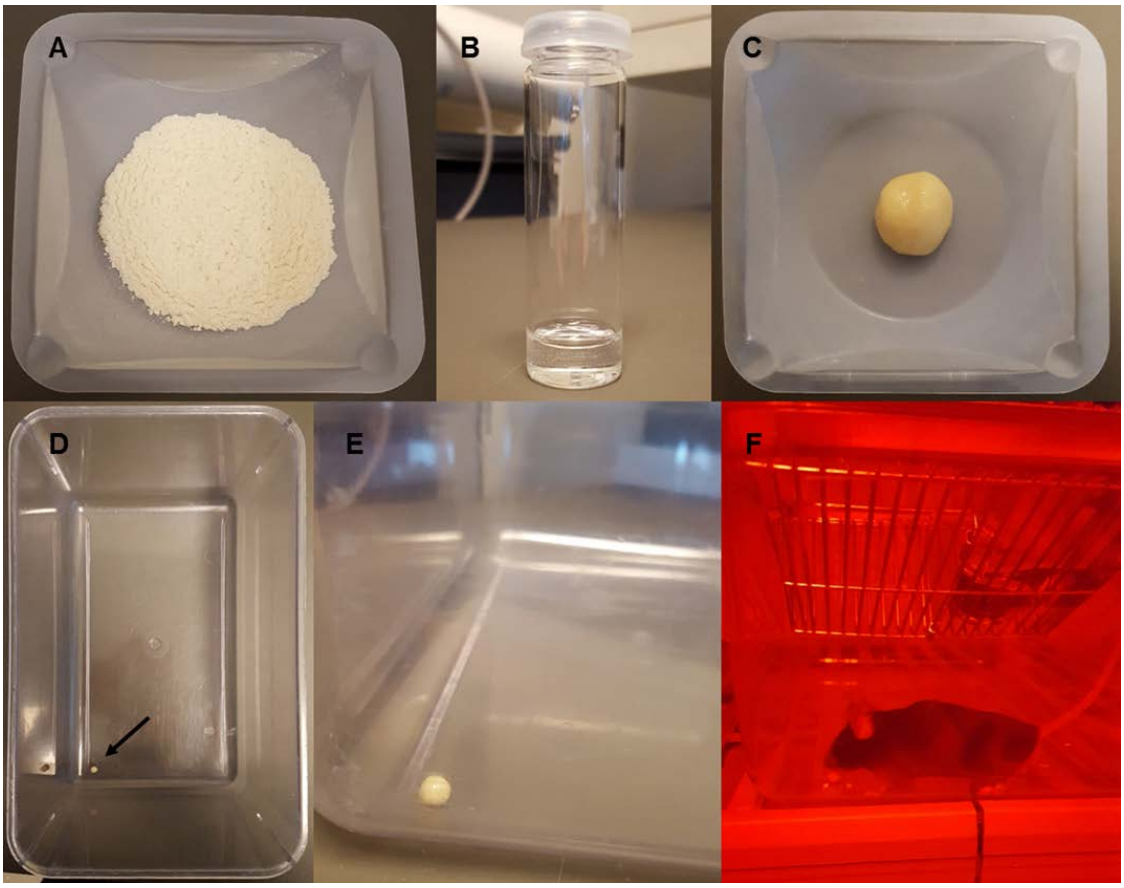

Figure 1. Preparation and serving of edible dough for mice using flour, sugar, salt, and glycerol (ratio = 30g : 4g : 1g : 20ml). A) Dough preparation begins by weighing and mixing all dry ingredients, B) followed by measuring and creating a glycerol solution using the NIDA-provided THC-ethanol solution (for THC dough) or just ethanol (for control dough). C) After pipetting the glycerol solution onto the dry ingredients, they are thoroughly combined by spatula and by hand to create a batch of dough (unique batch daily per THC dose) with the goal of uniform THC distribution. D) A single dough serving is portioned for each mouse (5 g/kg) and placed in a clean, empty mouse cage. E) Close-up of a single dough serving (125 mg for a 25 g mouse). F) Within the vivarium, mice are transferred to a clean, empty cage with their weight-adjusted dough serving during the first half of the dark cycle (red-light conditions) and allowed to consume dough with water available. Following the access period, mice are returned to their home cages, and the amount of uneaten dough is weighed to determine actual dough/dose consumed. Adapted from [Smoker, 2019b].

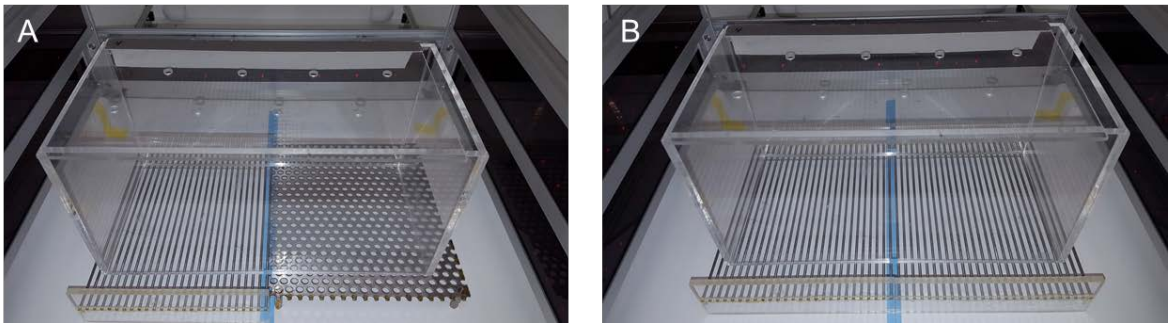

Figure 2. Images of place conditioning contexts. A) An example of the side-by-side arrangement of floor textures used during baseline and test sessions (grid left, hole right). B) An example of a single floor texture arrangement used during conditioning (all grid).

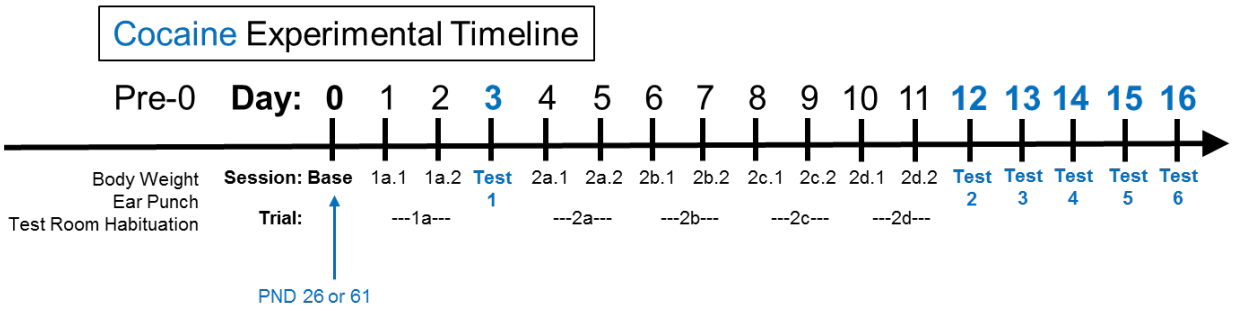

Figure 3. Experimental timeline for cocaine place conditioning.

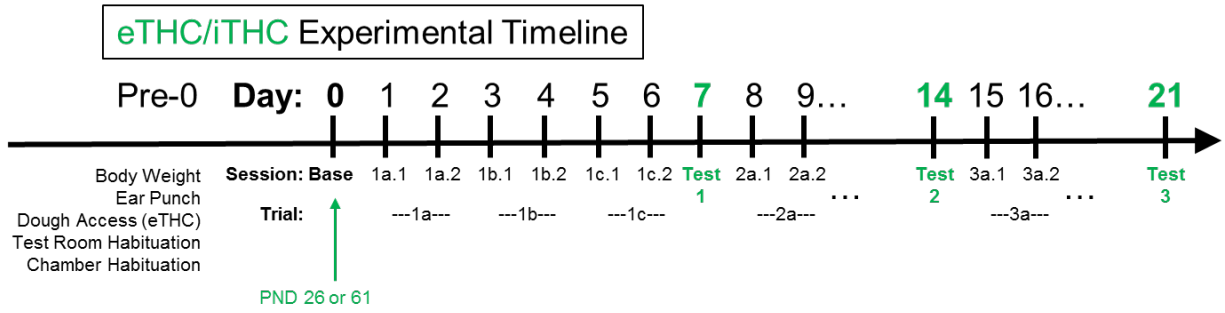

Figure 4. Experimental time line for THC place conditioning. (…) symbol indicates an analogous continuation over days of trials 2b-2c and 3b-3c as seen in trials 1b-1c.

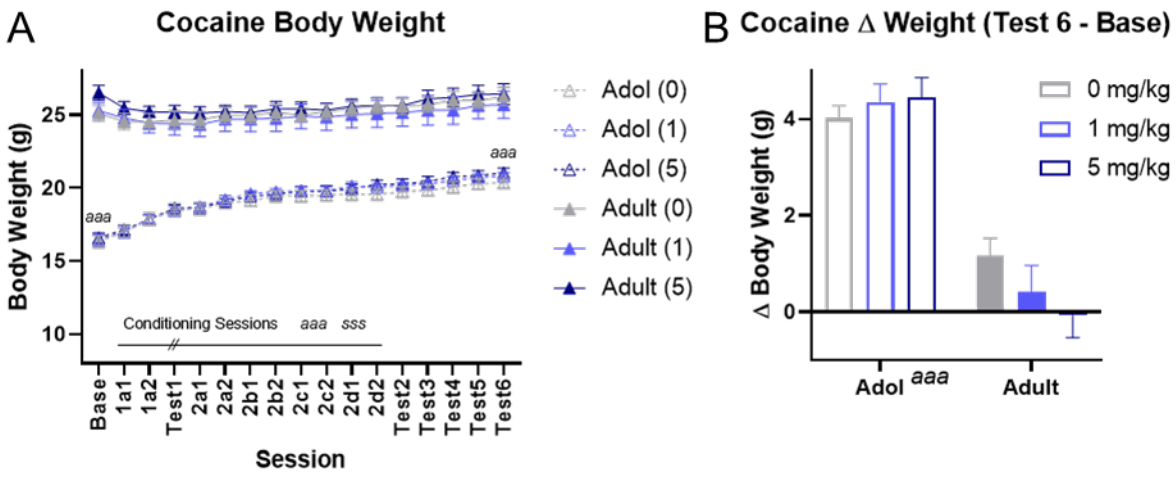

Figure 5. Body weight during cocaine place conditioning (Mean ± SEM). A) Body weight of mice across baseline, conditioning, and test sessions. Body weight was lower in adolescent mice at baseline, across conditioning sessions, and at the final test. Body weight also differed across conditioning sessions. $^{aaa}p < .001$ vs adult; $^{sss}p < .001$ main effect of conditioning session. B) Change in body weight from baseline to test 6. Adolescent mice gained more weight than adult mice. $^{aaa}p < .001$ vs adult.

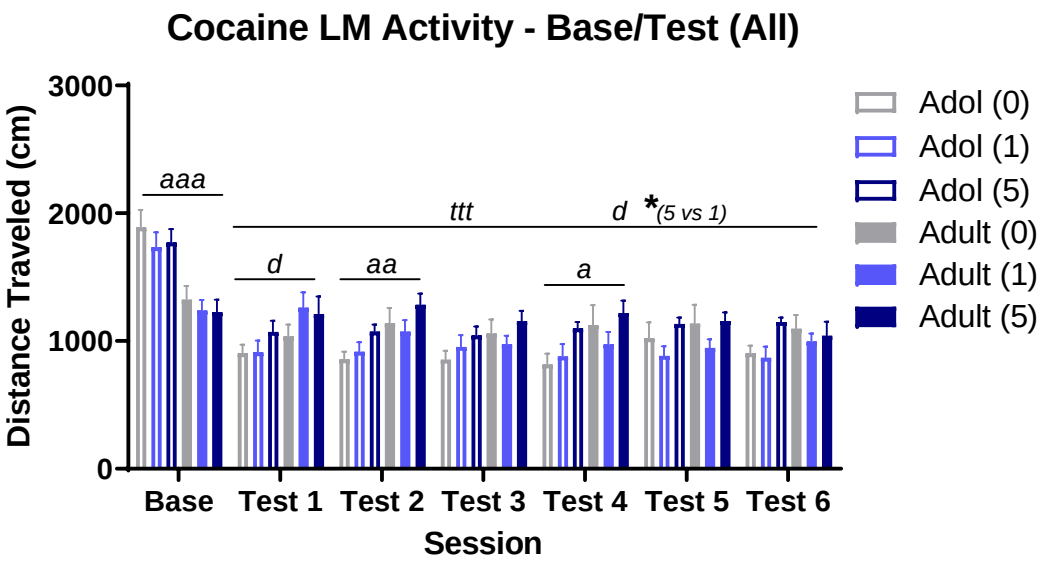

Figure 6. Locomotor activity during cocaine baseline and test sessions in a drug-free state (saline injections), (Mean ± SEM). Locomotor activity was elevated in adolescent mice at baseline but reduced in adolescent mice at tests 2 and 4. Cocaine caused an increase in locomotor activity across tests and specifically at test 1. Activity also varied as a function of test. ^{a} $p < .05$, ^{aa} $p < .01$, ^{aaa} $p < .001$ vs adult; ^{d} $p < .05$ main effect of dose, * $p < .05$ 5mg/kg vs 1mg/kg; ^{ttt} $p < .001$ main effect of test.

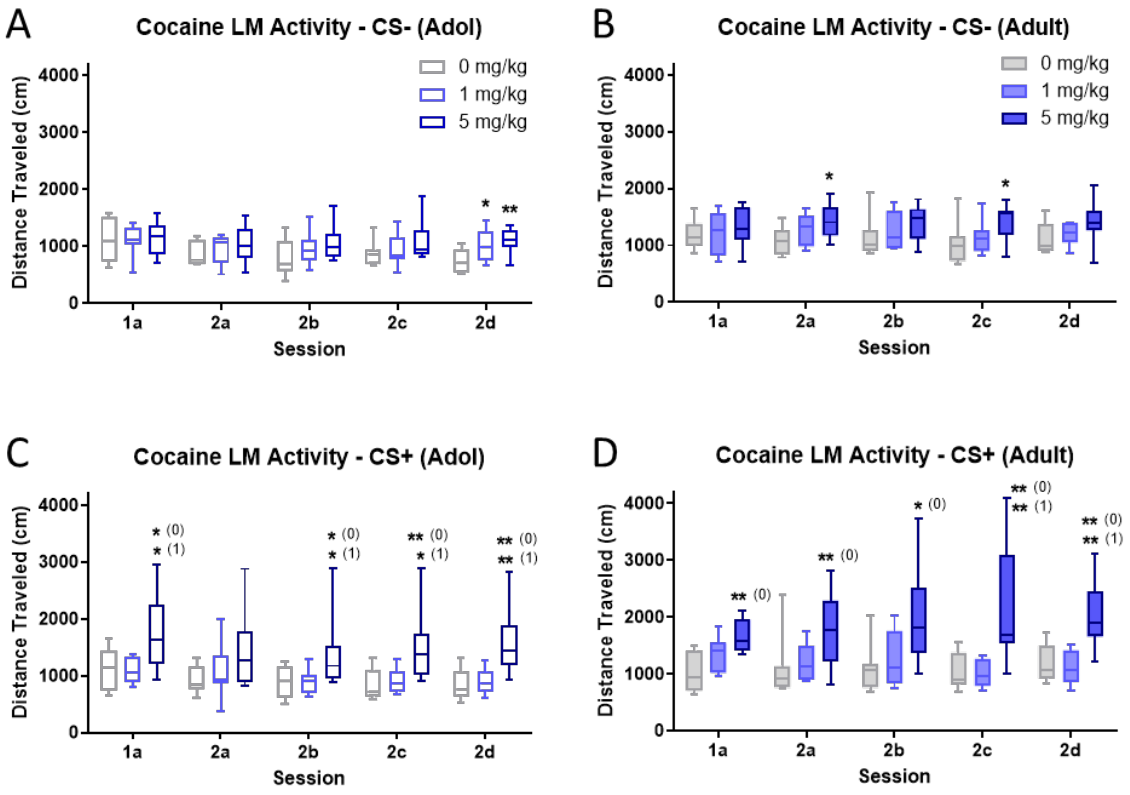

Figure 7. Boxplots of Locomotor activity during cocaine place conditioning sessions (Median). Cocaine increased activity of A) adolescent mice and B) adult mice on some saline (CS-) sessions. * $p < .05$, ** $p < .01$ vs 0mg/kg. Cocaine increased activity of C) adolescent mice on most and D) activity of adult mice on all cocaine (CS+) sessions. * $p < .05$, ** $p < .01$ vs (dose).

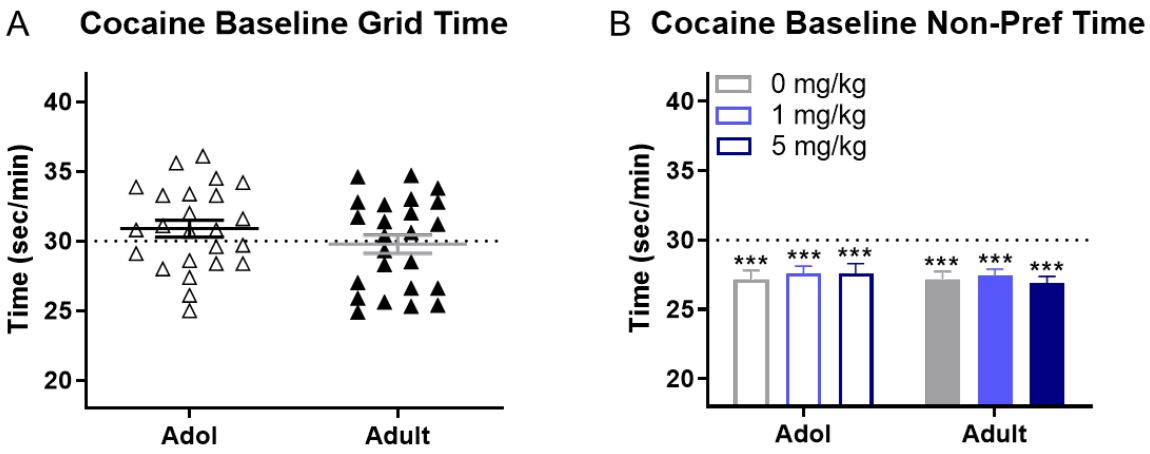

Figure 8. Time spent on the grid and non-preferred floors at baseline (Mean ± SEM). A) Time spent on the grid floor at baseline did not differ by age. B) Time spent on the non-preferred (cocaine-paired) floor at baseline did not differ by age or dose. *** $p < .001$ vs 30sec/min.

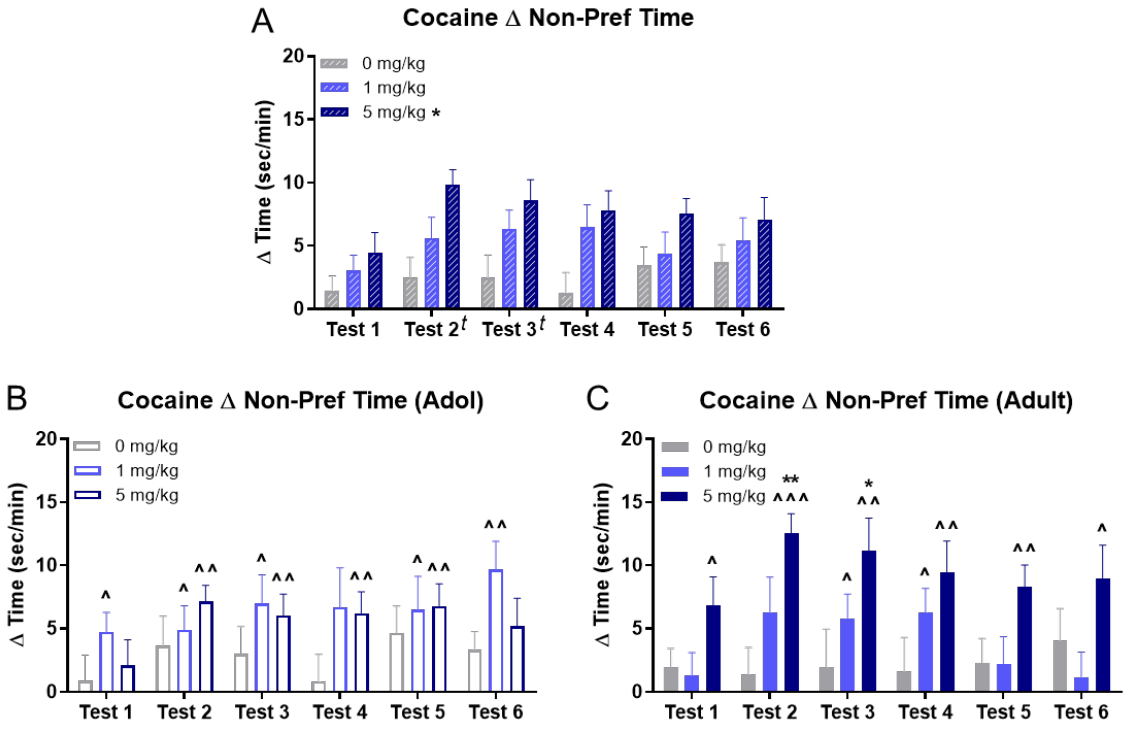

Figure 9. Change in time spent on the non-preferred floor (test – baseline) following cocaine place conditioning (Mean ± SEM). A) Cocaine increased the change in time spent on the non-preferred floor collapsed across age. * $p < .05$ vs 0mg/kg. Cocaine increased the change in time spent on the non-preferred side in B) adolescent mice and C) adult mice depending on analysis used. * $p < .05$, ** $p < .01$ vs 0mg/kg; ^ $p < .05$, ^^ $p < .01$ vs 0sec/min.

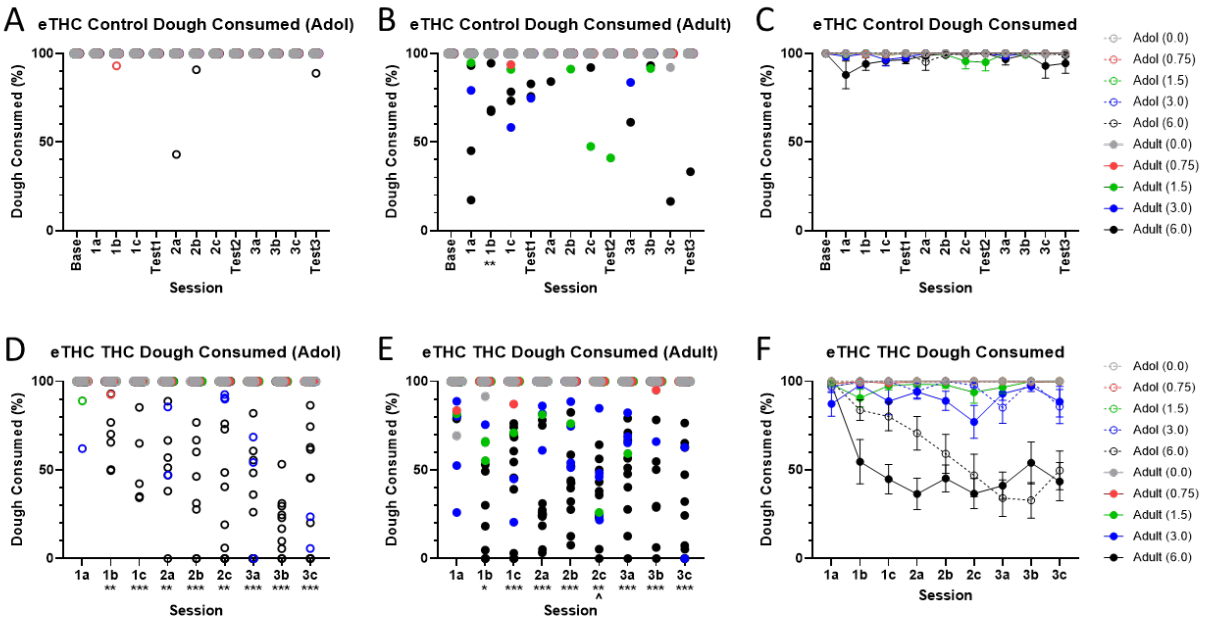

Figure 10. Amount of edible dough consumed by adolescent and adult mice. Amount of control dough consumed (%) by A) adolescent mice and B) adult mice. Control dough was very well consumed, but there was a reduction in consumption in adult mice on session 1b. ** $p < .01$ 6mg/kg vs all other doses. C) Consumption of control dough at the group level (Mean ± SEM). Amount of THC dough consumed by D) adolescent mice and E) adult mice. THC dough consumption decreased in both ages at the highest dose(s) provided beginning on session 1b. * $p < .05$, ** $p < .01$, *** $p < .001$ 6.0mg/kg vs all other doses within each age; ^ $p < .05$ 3.0mg/kg vs 0.0 and 0.75mg/kg. F) Consumption of THC dough at the group level (Mean ± SEM).

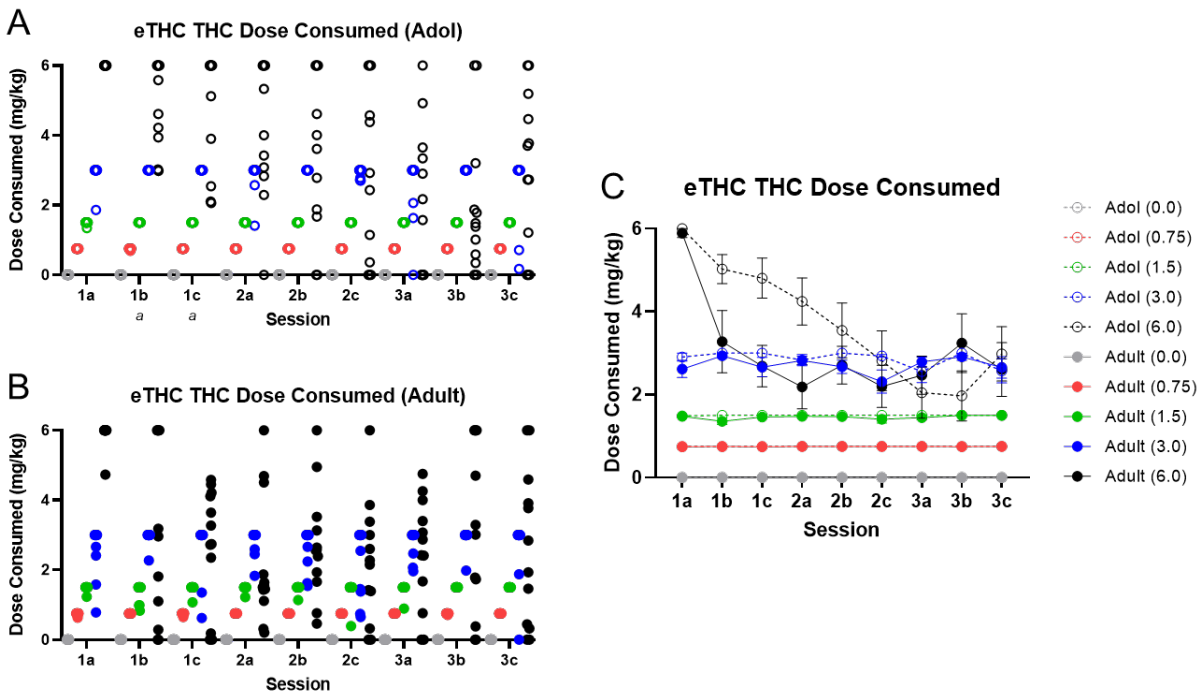

Figure 11. Edible THC dose consumed by individual mice based on dose provided for A) adolescent mice and B) adult mice. When provided the highest dose of edible THC, dose consumed by adolescent mice was greater on sessions 1b and 1c than dose consumed by adult mice. [a] $p < .05$ adolescent 6mg/kg vs adult 6mg/kg. C) Edible THC dose consumed at the group level (Mean ± SEM).

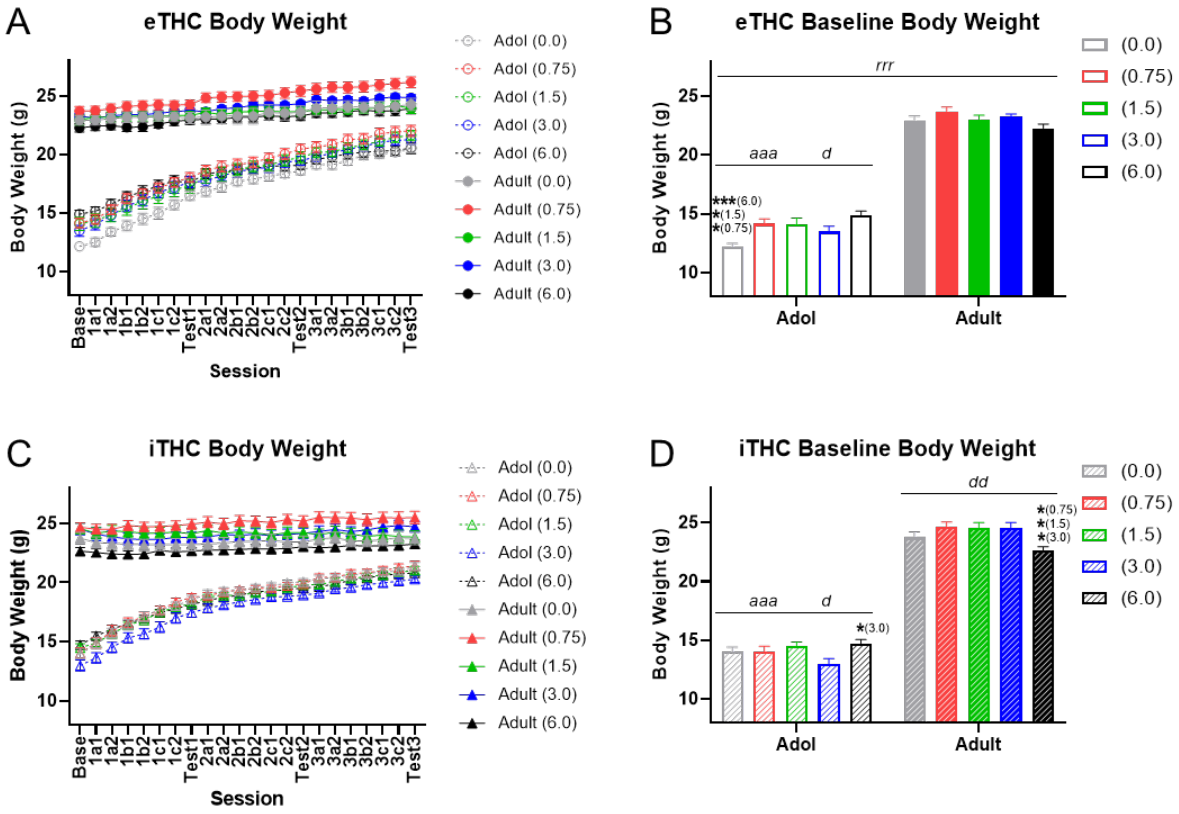

Figure 12. Body weight of mice at baseline and trajectory of body weight across THC place conditioning (Mean ± SEM). A) Body weight trajectory and B) baseline body weight of mice in the edible condition. At baseline, adolescent mice weighed less than adult mice, and adolescent weight differed by dose. C) Body weight trajectory and D) baseline body weight of mice in the injected condition. At baseline, adolescent mice weighed less than adult mice, and weight in both ages differed by dose. ^{aaa} $p < .001$ vs adult; ^{d} $p < .05$, ^{dd} $p < .01$ main effect of dose; ^{rrr} $p < .001$ main effect of ROA; * $p < .05$, *** $p < .001$ vs (dose).

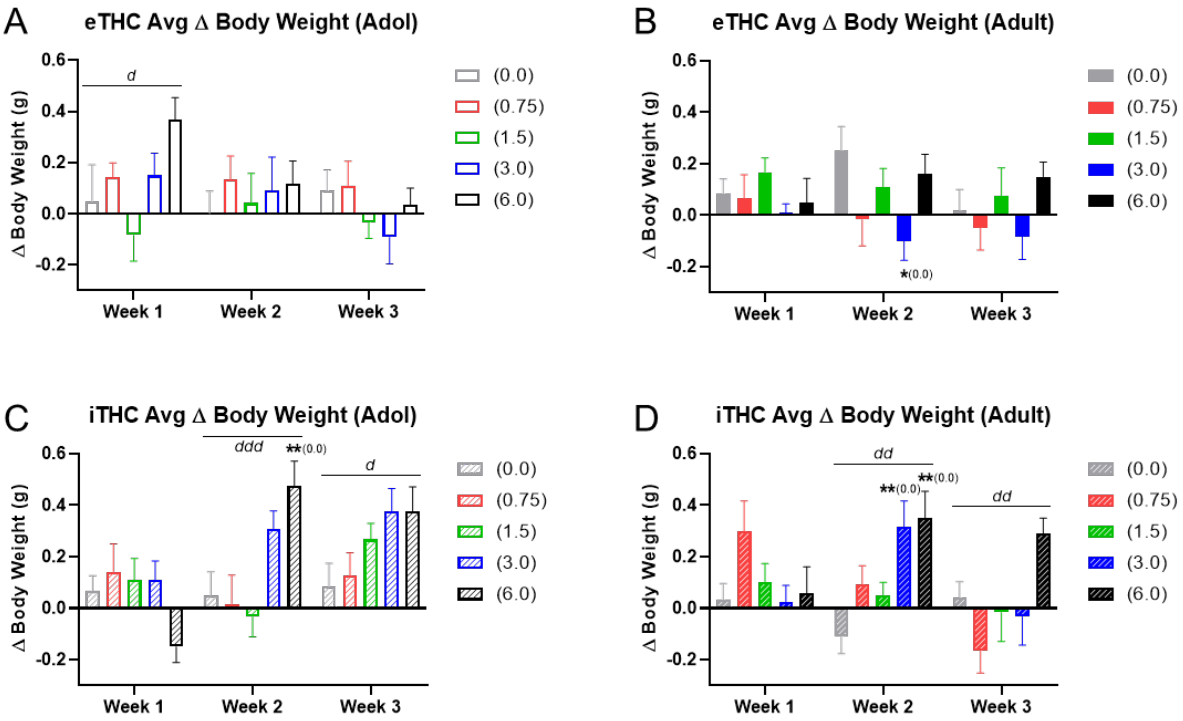

Figure 13. Weekly average of the difference (THC – control/vehicle) in the change in daily body weight in mice (Mean ± SEM). Average change in body weight for A) adolescent mice and B) adult mice in the edible condition. Change in weight differed by THC dose in adolescent mice in week 1. Average change in body weight for C) adolescent mice and D) adult mice in the injected condition. THC increased change in weight in both ages in weeks 2 and 3. ^{d} $p < .05$, ^{dd} $p < .01$, ^{ddd} $p < .001$ main effect of dose; ** $p < .01$ vs (dose).

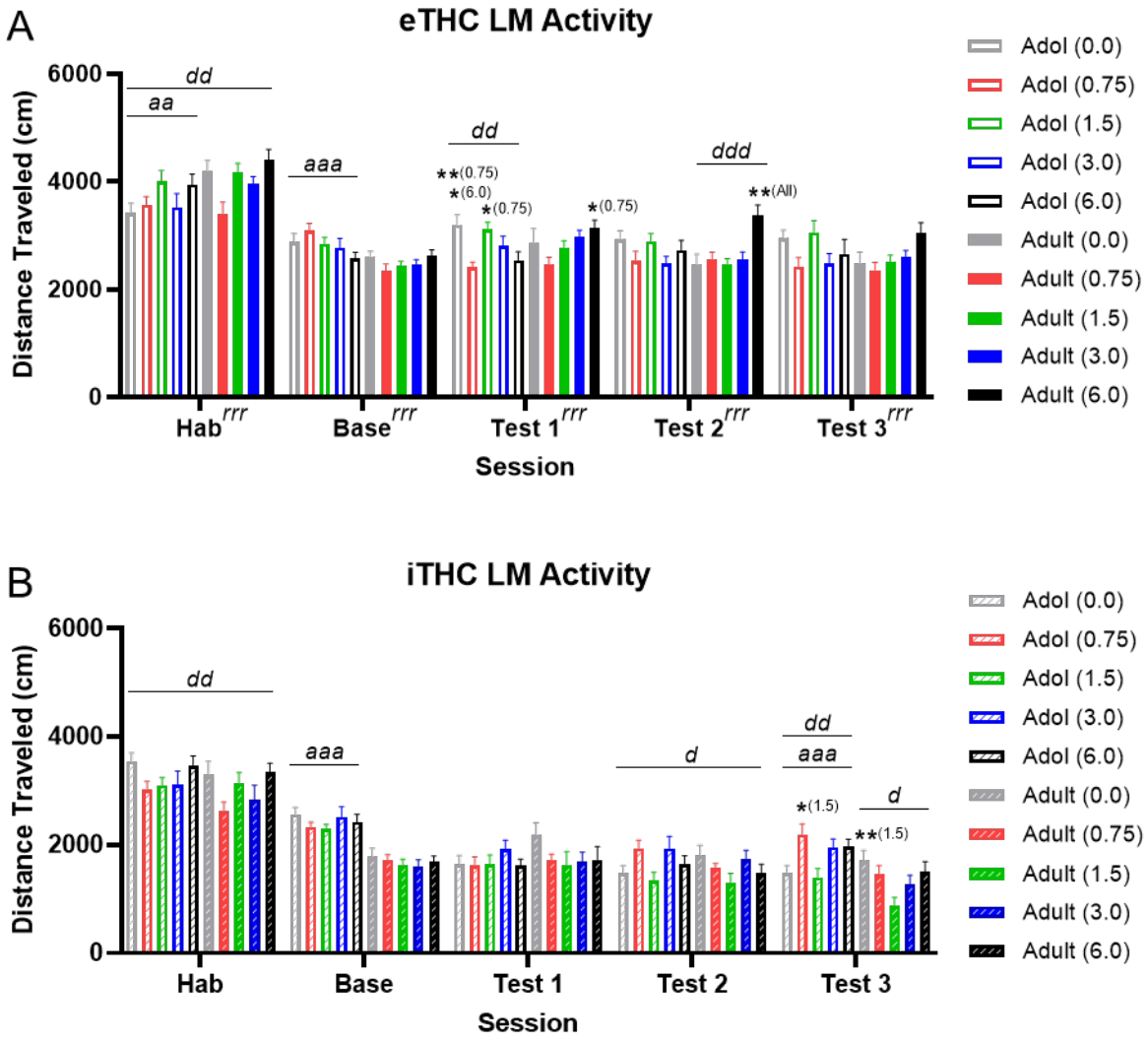

Figure 14. Locomotor activity during THC habituation, baseline, and test sessions in a drug-free state (Mean ± SEM). A) Locomotor activity for adolescent and adult mice in the edible condition. Adolescent mice were less active at habituation but more active at baseline. Locomotor activity differed by THC dose across ages at habituation, in adolescent mice at test 1, and in adult mice at test 2. B) Locomotor activity for adolescent and adult mice in the injected condition. Adolescent mice were more active at baseline and at test 3. Locomotor activity differed by THC dose across ages at habituation, across ages at test 2, and within each age at test 3. Mice in the edible condition were more active during all sessions. ^{aa} $p < .01$, ^{aaa} $p < .001$ vs adult; ^{d} $p < .05$, ^{dd} $p < .01$, ^{ddd} $p < .001$ main effect of dose; ^{rrr} $p < .001$ main effect of ROA; * $p < .05$, ** $p < .01$ vs (dose).

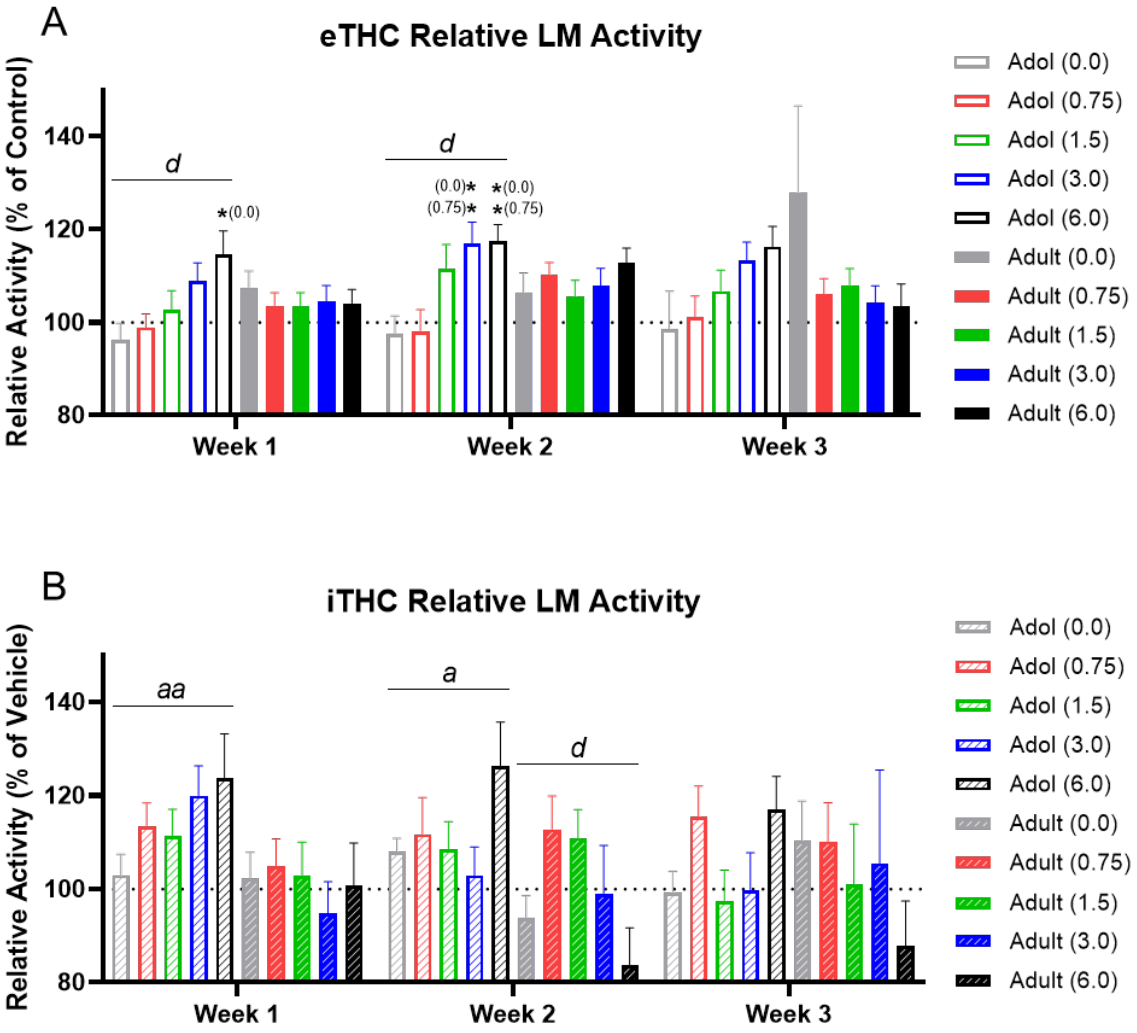

Figure 15. Weekly average of relative locomotor activity (THC session as % of control/vehicle session) per trial (Mean ± SEM). A) Average relative activity in adolescent and adult mice in the edible condition. THC increased activity in adolescent mice in weeks 1 and 2. B) Average relative activity in adolescent and adult mice in the injected condition. Adolescent mice displayed more relative activity than adult mice in weeks 1 and 2, and relative activity differed by THC dose in adult mice in week 2. ^{a} $p < .05$, ^{aa} $p < .01$ vs adult; ^{d} $p < .05$ main effect of dose; * $p < .05$ vs (dose).

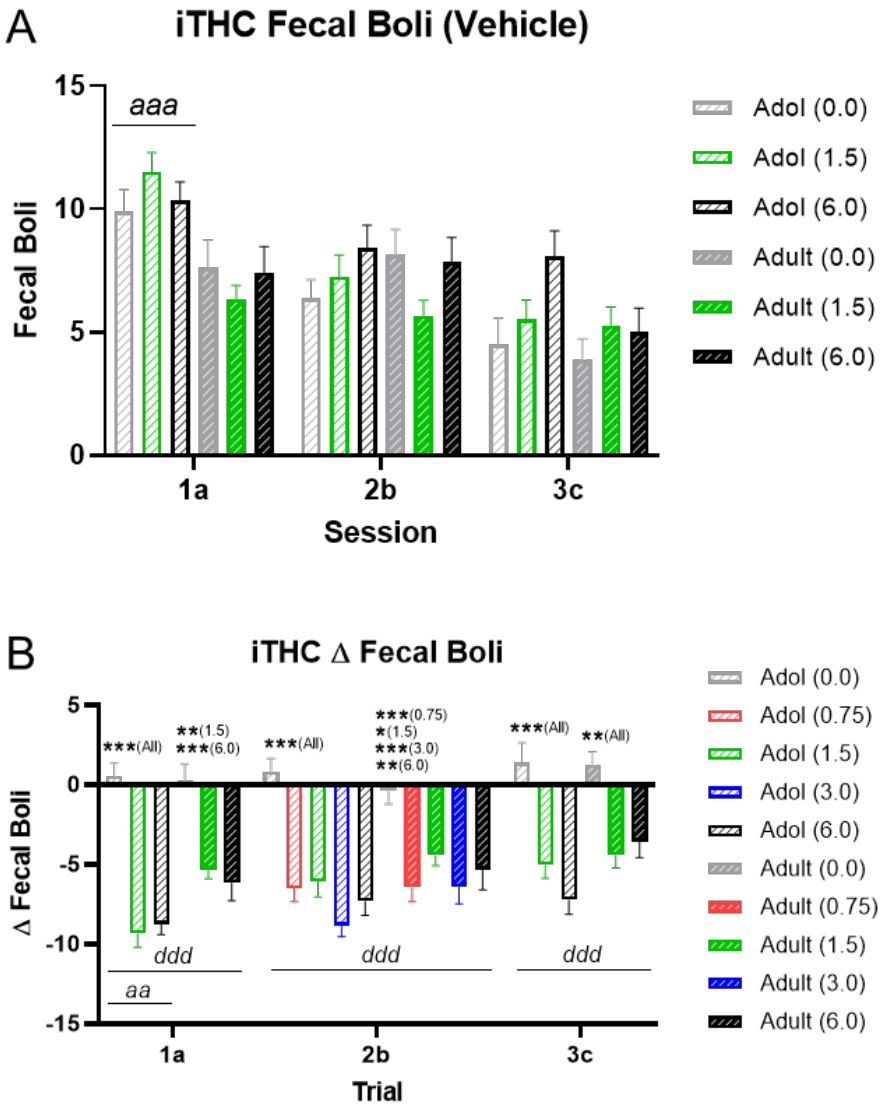

Figure 16. Fecal boli produced during conditioning for a subset of mice in the injected condition (Mean ± SEM). A) Fecal boli produced by adolescent and adult mice during vehicle sessions. Adolescent mice show an increase in boli produced during session 1a. B) Difference in fecal boli produced (THC session – vehicle session) for a given trial. THC reduced the amount of boli produced at all doses for all trials in both ages. ^{aa} $p < .01$, ^{aaa} $p < .001$ vs adult; ^{ddd} $p < .001$ main effect of dose; * $p < .05$, ** $p < .01$, *** $p < .001$ vs (dose).

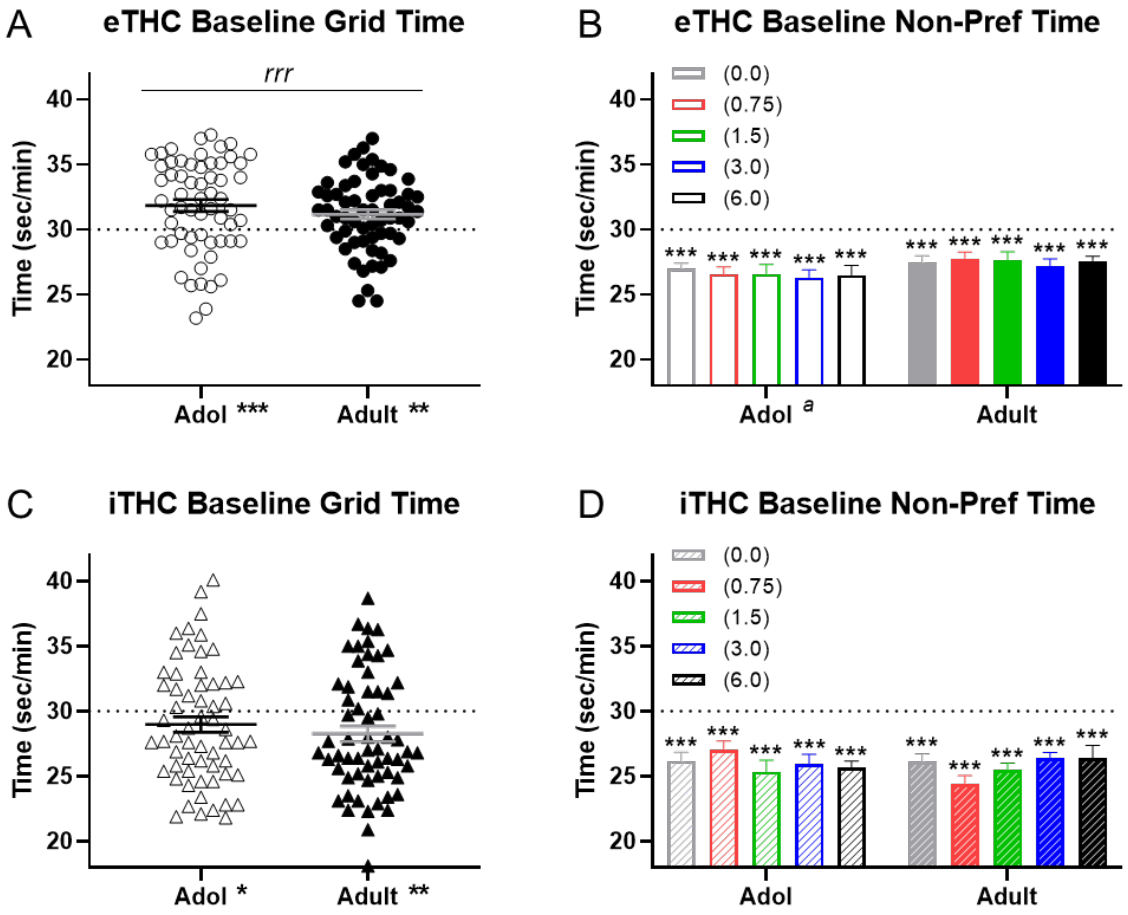

Figure 17. Time spent on the grid and non-preferred floor at baseline (Mean ± SEM). Time spent on the A) grid floor and B) non-preferred floor by adolescent and adult mice in the edible condition. Mice of both ages showed a preference for the grid floor. Time spent on the non-preferred (THC-paired) floor at baseline was lower in adolescent mice but did not differ by dose. Time spent on the C) grid floor and D) non-preferred floor by adolescent and adult mice in the injected condition. Mice of both ages showed a preference for the hole floor. Time spent on the non-preferred floor at baseline did not differ by age or dose. Baseline floor preference was opposite for mice in the edible vs injected condition. [a] $p < .05$ vs adult; [rrr] $p < .001$ main effect of ROA; * $p < .05$, ** $p < .01$, *** $p < .001$ vs 30sec/min.

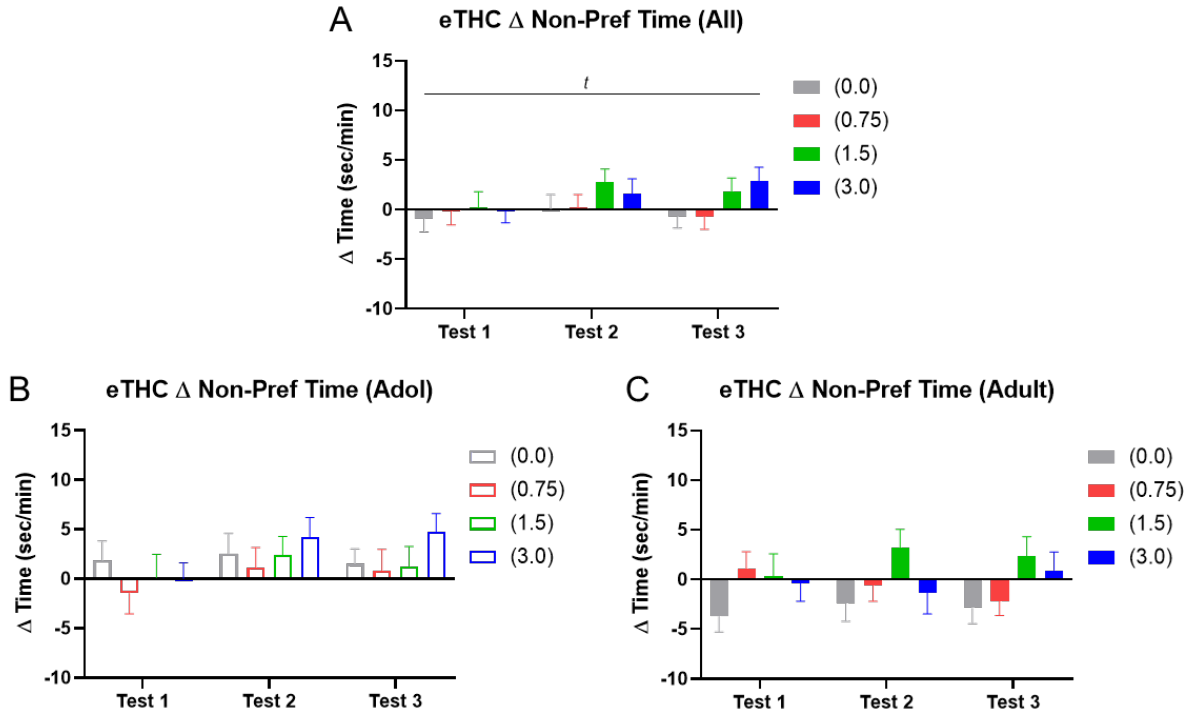

Figure 18. Change in time spent on the non-preferred floor (test – baseline) following edible THC place conditioning (Mean ± SEM). Change in time on the non-preferred floor for A) all mice, B) adolescent mice, and C) adult mice. Change in time spent on the non-preferred floor differed across tests in all mice but did not differ by dose at any test in any age. $^{t}p < .05$ main effect of test. Note: mice provided 6.0mg/kg THC as well as mice provided 3.0mg/kg THC which ever consumed 0% of dough, or which ever averaged < 50% dough consumption for any single week were excluded.

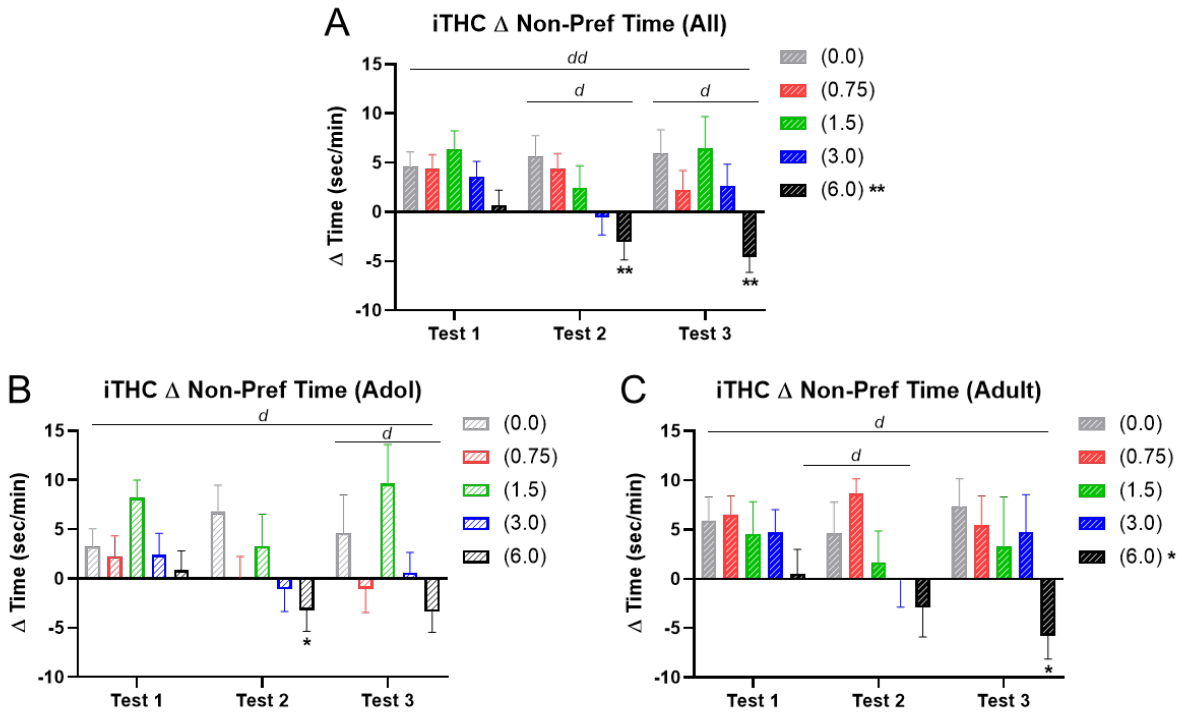

Figure 19. Change in time spent on the non-preferred floor (test – baseline) following injected THC place conditioning (Mean ± SEM). Change in time on the non-preferred floor for A) all mice, B) adolescent mice, and C) adult mice. A) THC decreased time spent on the non-preferred floor across both ages overall and at tests 2 and 3. B/C) Change in time spent on the non-preferred floor differed by THC dose across tests and at some individual tests in both ages. $^{d}p < .05$, $^{dd}p < .01$ main effect of dose; * $p < .05$, ** $p < .01$ vs 0mg/kg.

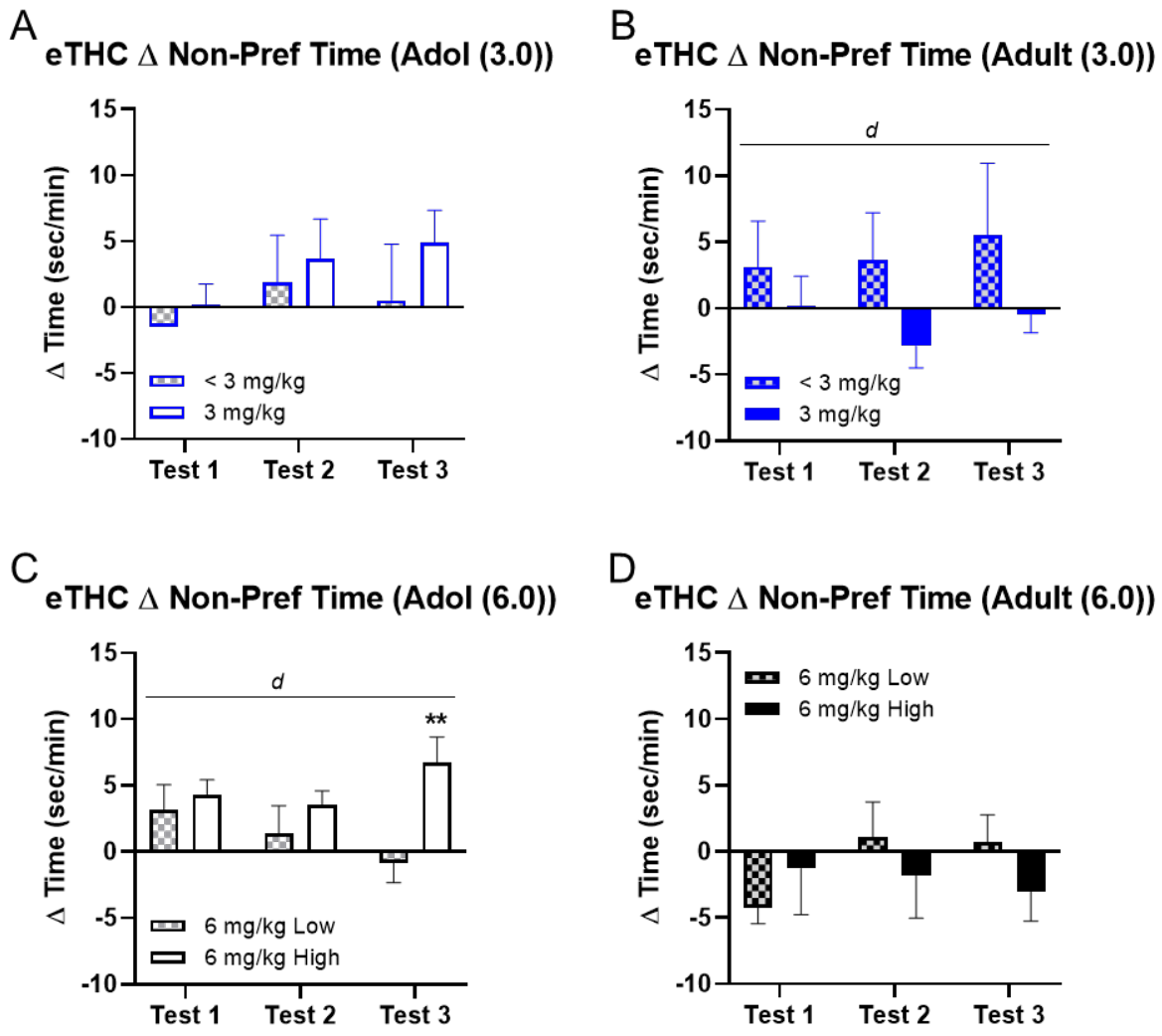

Figure 20. Change in time spent on the non-preferred floor following edible THC place conditioning based on THC dose consumed (Mean ± SEM). Change in time spent on the non-preferred floor in A) adolescent mice and B) adult mice which consumed an average of 3.0mg/kg or < 3.0mg/kg per week when provided a 3.0mg/kg edible THC dose. Adult mice consuming < 3.0mg/kg show a relative increase in time spent on the non-preferred floor. Change in time spent on the non-preferred floor in C) adolescent mice and D) adult mice based on a median split within age of the highest and lowest average dose consumed per week when provided a 6.0mg/kg edible THC dose. Adolescent mice consuming higher amounts of THC show a relative increase in time spent on the non-preferred floor. [d] $p < .05$ main effect of dose (consumed); ** $p < .01$ vs 6mg/kg Low.

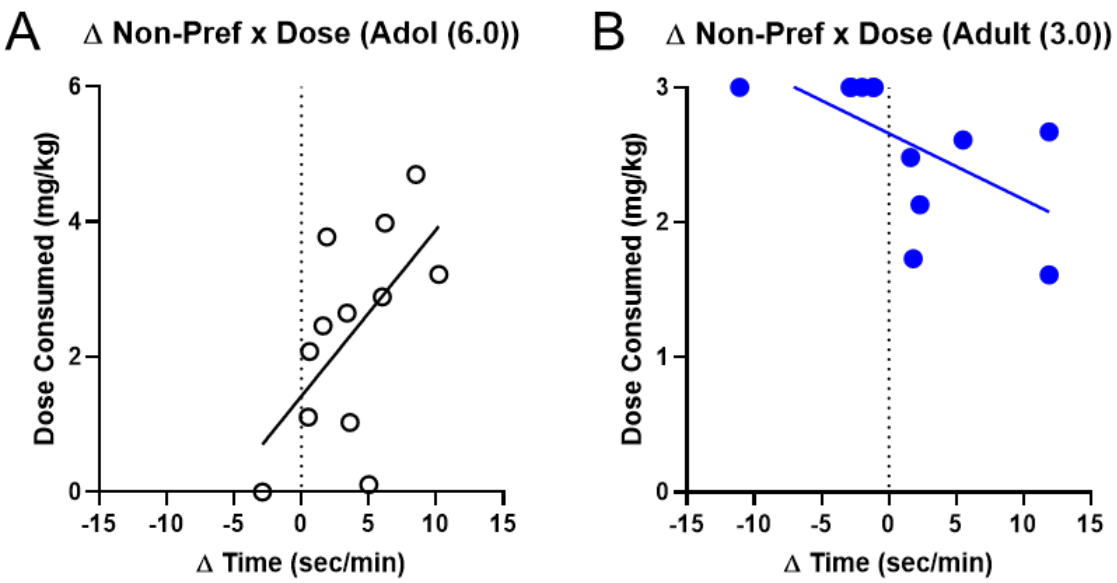

Figure 21. Example relationships between change in time spent on the non-preferred floor and subsequent edible THC dose consumed. A) Change in time spent on the non-preferred floor at test 1 is predictive of average edible THC dose consumed in week 3 in adolescent mice provided 6.0mg/kg. B) Change in time spent on the non-preferred floor at test 1 is predictive of average edible THC dose consumed in week 2 in adult mice provided 3.0mg/kg.